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Inflammation related gene expression by lipid oxidation derived products in the progression of atherosclerosis

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Abstract

Vascular areas of atherosclerotic development persist in a state of inflammation, and any further inflammatory stimulus in the subintimal area becomes a pro-atherogenic response; this alters the behavior of the artery wall cells and recruits further inflammatory cells. In association with the inflammatory response, oxidative events are also involved in the development of atherosclerotic plaques. It is now unanimously recognized that lipid oxidation-derived products are key players in the initiation and progression of atherosclerotic lesions.

Oxidized lipids, derived from oxidatively modified low-density lipoproteins (LDL) which accumulate in the intima, strongly modulate inflammation-related gene expression, through involvement of various signaling pathways. In addition, considerable evidence supports a pro-atherogenic role of a large group of potent bioactive lipids called eicosanoids, which derive from oxidation of arachidonic acid, a component of membrane phospholipids. Of note, LDL lipid oxidation products might regulate eicosanoid production, modulating the enzymatic degradation of arachidonic acid by cyclooxygenases and lipoxygenases; these enzymes might also directly contribute to LDL oxidation.

This review provides a comprehensive overview of current knowledge on signal transduction pathways and inflammatory gene expression, modulated by lipid oxidation derived products, in the progression of atherosclerosis.

Keywords: atherosclerosis; oxidized LDL; cell signaling; inflammation; oxysterols; aldehydes; core-aldehydes; oxidized phospholipids; prostanoids; leukotrienes.

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Introduction: modulation of inflammatory signaling by oxidized lipid products

It is generally accepted that vascular areas of atherosclerotic progression are in a state of persistent inflammation [1,2]. As a consequence, any further inflammatory stimulus in the subintimal area automatically becomes a pro-atherogenic stimulus, altering the behavior of the intrinsic cells of the artery wall, and recruiting further inflammatory cells that interact to promote lesion formation and complications. Of note, leukocytes are present even in the very earliest fatty streak lesions [3-8]. The association of hypercholesterolemia with atherosclerosis is also well established [9,10]. Given that numerous oxidative events are associated with the development of atherosclerotic plaques [11], it is now accepted that oxidized low-density lipoproteins (oxLDLs), which accumulate in the intima, play a major role in the initiation and promotion of fatty streaks and fibrotic plaques [12,13]. In addition, considerable evidence supports a pro-atherogenic role of oxLDLs. The biological effects of oxLDLs are mediated through signaling pathways, especially involving receptors, protein kinases, and activation of transcription factors, which in turn stimulate the expression of genes involved in oxidative stress and the inflammatory response during generation of the atherosclerotic plaque [14,15]. Indeed, oxidative stress and inflammation go hand-in-hand, because oxidative stress induces the production of inflammatory cytokines, and the cytokines in turn induce free radical production.

The main lipid oxidation products present in oxLDLs, and which may be responsible for inflammatory processes, are oxysterols and cholesteryl ester oxidation products, lipid-derived free aldehydes and oxidized phospholipids. Another quantitatively important component of oxLDLs, alongside the oxidized lipid products proper, are the lysophospholipids, hydrolytic derivatives of oxidized phospholipids. Moreover, among the oxidized lipid products implicated in atherogenesis, there is a large group of potent bioactive lipids, known as prostanoids, isoprostanoids, and leukotrienes, which derive from the oxidation of arachidonic acid, a component of membrane phospholipids. All these compounds have been directly linked to the induction and propagation of

monocytic subendothelial accumulation, and to other inflammatory reactions associated with chronic vascular inflammation. Macrophages in the atheroma may also have a pro-inflammatory array of functions, characteristic of M1 macrophages, which produce high levels of effectors such as cytokines, chemokines, growth factors, adhesion molecules, and other inflammatory molecules.

Conversely, accumulating data suggest that oxidized lipids may also have anti-inflammatory potential, and could, in certain cases, act as inhibitors of the nuclear factor- κ B (NF- κ B)-dependent pro-inflammatory cascade [16].

This review aims to comprehensively summarize current knowledge on the signal transduction pathways, and on inflammatory gene expression modulated by lipid oxidation derived products, in the progression of atherosclerosis.

Oxysterols

Oxysterols are 27-carbon atom molecules resulting from non-enzymatic or enzymatic oxidation of cholesterol, which may either originate in the blood, cells and tissues, or may derive from the diet. Several reviews have comprehensively discussed the sources of major biological oxysterols and the routes for their formation [17-24]. In general, biological oxysterols can be divided into two main groups: 1) those oxygenated in the sterol ring, mainly at position 7 (e.g. 7 α / β -hydroxycholesterol, 7 α / β -hydroperoxycholesterol, and 7-ketcholesterol), which have non-enzymatic origin; 2) those oxygenated on the side-chain (e.g. 24S-hydroxycholesterol, 25-hydroxycholesterol, and 27-hydroxycholesterol) which usually have enzymatic origin. Some oxysterols, however, can be produced by either non-enzymatic or enzymatic routes: these include 25-hydroxycholesterol and 7 α -hydroxycholesterol.

Oxysterols are a large and diverse group of compounds with a multiplicity of biological activities; they have been found at increased levels in the plasma of patients with cardiovascular

diseases, and within atherosclerotic lesions. Depending on the nature and location of the oxygen substitution, oxysterols have distinct effects on the biophysical properties of cell membranes, with which they interact faster than does cholesterol, due to higher polarity and poorer membrane packing. Besides the impact of oxysterols on the biophysical properties of membranes, endogenous cellular oxysterols are thought to drive important functions, by interacting with receptor proteins [23,25,26]. In this connection, oxysterols not only participate in basic metabolic processes, but are also involved in signaling pathways leading to disease development, through induction of inflammation, apoptosis, and fibrosis.

Being multifunctional molecules, oxysterols appear to exert a number of pro-inflammatory effects during the progression of atherosclerosis; however, it is extremely difficult to study all routes involved, and the molecular mechanisms whereby oxysterols induce inflammation at the transcriptional level are not yet fully elucidated. Oxysterol-dependent gene expression is probably regulated by various different transcriptional regulators. In this connection, activation of liver X receptors (LXR) can only be induced by a small number of pro-inflammatory oxysterols, acting as natural ligands [27], while it has been demonstrated that NF- κ B or activator protein-1 (AP-1) nuclear binding can be induced by a biologically representative oxysterol mixture [28,29]. Similarly, the oxysterol-induced metabolic pathways at the translational and post-translational levels are still unclear, although mitogen-activated protein kinases (MAPKs) are implicated [28-31]. MAPKs include extracellular signaling-regulated kinase (ERK), c-Jun N-terminal kinase (JNK), and p38 MAPK, which are involved in several cellular functions, ranging from proliferation to differentiation and apoptosis, and which thus play an important role in the pathogenesis of cardiac and vascular disease [32].

Oxysterol-mediated up-regulation of chemokines, inflammatory cytokines and metalloproteinases

Oxysterols have been shown to induce the expression of various key inflammatory molecules. They stimulate not only the adhesion of leukocytes to the arterial endothelium, but also their transmigration to sub-intimal spaces, especially by up-regulating chemotactic cytokines such as monocyte chemotactic protein-1 (MCP-1) and interleukin-8 (IL-8).

Up-regulation of MCP-1 has been reported in U937 promonocytic cells stimulated by a biologically relevant oxysterol mixture, through the ERK and NF- κ B pathways [28]. Macrophages isolated from human atherosclerotic plaques produce IL-8, and oxysterols appear to be involved in production of this cytokine [33]; IL-8 is up-regulated in human monocytes/macrophages by various oxysterols, including 25-hydroxycholesterol (25-OH), 24-hydroxycholesterol (24-OH), 7 β -hydroxycholestetrol (7 β -OH), 5 α ,6 α -epoxycholesterol (α -EPOX), 7-ketocholesterol (7-K), and cholestan-3 β ,5 α ,6 β -triol (TRIOL) [34-37]. Using calcium channel blockers, it has been shown that both 7 β -OH-induced and 25-OH-induced IL-8 secretion by THP-1 cells involves calcium-dependent activation of c-fos (AP-1) via the ERK1/2 signaling pathway [37].

The oxysterols 25-OH, 7 β -OH and 7-K have been found to stimulate IL-8 expression in human macrophages, independently of Toll-like receptor (TLR) signaling, although this pathway has been shown to contribute to the inflammatory events occurring during atherosclerosis progression [36]. IL-8 production is also induced in Caco2 cells by treatment with 25-OH followed by IL-1 β stimulation [38]. The chemotactic cytokine IL-8 might be pro-atherogenic, acting not only by recruiting leukocytes, but also by inhibiting expression of the tissue inhibitor of metalloproteinase-1 (TIMP-1). This event causes an imbalance in the metalloproteinases (MMPs)/TIMPs activity ratio, with a consequent excessive degradation of extracellular matrix components, followed by destabilization and eventual rupture of the atherosclerotic plaque [39]. In this connection, in human promonocytic U937 cells it is reported that an oxysterol mixture of composition similar to that detectable in advanced human carotid plaques induces expression and synthesis of MMP-9, without affecting its endogenous inhibitors TIMP-1 and TIMP-2. Using

antioxidants, or specific inhibitors, or siRNAs, it has been demonstrated that the oxysterol mixture induces MMP-9 expression through: i) over-production of reactive oxygen species (ROS), likely by NADPH oxidase and mitochondria, ii) up-regulation of MAPKs signaling pathways via protein kinase C (PKC), iii) up-regulation of AP-1 and NF- κ B DNA binding [29].

Further, in human monocytic cells, 7 β -OH and 25-OH, but also to a lesser extent 7-K, are potent *in vitro* inducers of MCP-1, IL-8, IL-1 β , tumor necrosis factor- α (TNF- α), macrophage inflammatory protein-1 β (MIP-1 β), as well as of other inflammatory molecules [31]. The same study demonstrated that IL-8 secretion was associated with activation of the ERK1/2 signaling pathway [31]. The oxysterols 7-K and 25-OH have also been observed to enhance IL-1 β , IL-6, IL-8 and TNF- α mRNA and secretion levels, in a dose-dependent manner, although to different extents. These effects were associated with increased ROS production, through enhanced expression of NADPH oxidase; net phosphorylation of MAPKs and NF- κ B activation also occurred [40].

Up-regulation of IL-1 β is another important event, because this cytokine increases the surface expression of endothelial adhesion molecules, by facilitating inflammatory cells' attachment to the artery endothelium. Expression and synthesis of IL-1 β were found to be stimulated by 25-OH in human macrophages, through involvement of LXR, as well as, but less strongly, by 27-hydroxycholesterol (27-OH). 25-OH was also capable of potentiating lipopolysaccharide (LPS)-induced IL-1 β secretion [41]. IL-1 β secretion was also markedly induced by 7 β -OH, 7-K and 7 α -hydroxycholesterol (7 α -OH) in human promonocytic cells U937 and U4 [35,42] and in human umbilical vein endothelial cells (HUVECs) [43].

Increased levels of IL-6 have also been found in human macrophages stimulated with 25-OH or 27-OH [41]. IL-6 secretion was increased by 7-K treatment in human aorta smooth muscle cells (SMCs) through MAPK activation, in particular by p38 MAPK, but also via JNK pathways [44]. Furthermore, IL-6 regulates the expression of other inflammatory cytokines, such as IL-1 and TNF- α [45].

Expression of TNF- α is stimulated by 25-OH in macrophages, only when the cells were co-treated with 9-cis-retinoic acid, through heterodimerisation of the LXR [46]. Production of the pro-inflammatory cytokines TNF- α and IL-1 is also induced by 25-OH in adherent human peripheral blood mononuclear leukocytes, through phosphorylation of p38 MAPK [47]. Further, synthesis of TNF- α has been reported in human peripheral monocytes and in monocytic THP-1 cells treated with 22-hydroxycholesterol (22-OH) [46]. Another effect of 7-K and 25-OH, which may enhance the inflammatory response, is inhibition of secretion of IL-10, a key anti-inflammatory cytokine, in SMCs [48]. Treatment of vascular SMCs with 7-K enhances synthesis of the vascular endothelial growth factor (VEGF) [49].

Up-regulation of transforming growth factor β 1 (TGF β 1), the main pro-fibrogenic cytokine contributing to the progression of inflammation and fibrosclerosis within the damaged arterial wall, has also been shown to be exerted by a biologically representative oxysterol mixture, in J774.A1 murine macrophagic cell line [50]. In particular, production of TGF β 1 plays a pivotal role in SMC chemoattraction and differentiation into myofibroblast-like cells. 25-OH has also been shown to increase the expression of basic fibroblast growth factor (bFGF), a strong mitogenic and fibrogenic cytokine, in SMC [51].

Induction of the endothelial inflammatory phenotype

Oxysterols can contribute to increased expression of several adhesion molecules, which play key roles in endothelial adhesion and transmigration of leukocytes. Oxysterols, such as 7-K, 7 α -OH and 7 β -OH, increase the level of adhesion molecules involved in the recruitment of immunocompetent cells, such as vascular cell adhesion molecule-1 (VCAM-1), intercellular adhesion molecule-1 (ICAM-1) and E-selectin, in HUVECs [43,52,53]. Moreover, incubation with 7-K markedly induces ROS-dependent secretion of VCAM-1 in human aortic endothelial cells

(ECs) and U937 cells [54]. It has also been shown that 25-OH can augment eicosanoid release from cultured ECs and increase endothelial-leukocyte interaction by up-regulating VCAM [55].

It is known that endothelial dysfunction plays a central role in atherosclerosis, and 7-K has been found to increase expression of the actin-binding protein profilin-1 in aortic ECs. Up-regulation of the pro-atherogenic protein profilin-1 appears to occur through activation of a transduction molecule known as signal transducer and activator of transcription 3 (STAT3), which requires Janus kinase 2 (JAK2) and tyrosine 394 phosphorylation of oxysterol-binding protein-1 (OSBP-1) [56]. Again, oxysterols can be inserted into the cell membrane, where they modify the functions of membrane-bound proteins: 7-K has been found to induce endothelial dysfunction by disrupting ion transport by Na/K-ATPase, and to perturb the membrane structure in human ECs. For these reasons, this oxysterol-induced effect may contribute to the rigidity of plasma membranes [57].

Monocyte differentiation and foam cell formation

Oxysterols contribute to sustaining inflammation, by favoring monocyte differentiation into macrophages. It has been shown that a biologically compatible mixture of oxysterols up-regulates the expression and synthesis of the scavenger receptor CD36, with consequent uptake of oxLDLs, involving PKC- δ , ERK, and the peroxisome proliferator-activated receptor γ (PPAR γ) pathways [58]. Indeed, the monocyte differentiation into macrophages observed in oxLDL-treated THP-1 cells has been attributed to its major oxysterols, 7 β -OH and 7-K [59]. Foam cell formation, a key process during the development of atherosclerosis, which leads to vascular inflammation, is also sustained by TLR4: deficiency of TLR4 significantly reduces macrophage lipid accumulation in vascular lesions [60]. Furthermore, the common Asp299Gly TLR4 polymorphism, which causes loss of function, is associated with lower plasma levels of pro-inflammatory cytokines, adhesion molecules and acute-phase proteins, and a decreased atherosclerosis risk [61].

The accumulation of lipids in the macrophages present at the subendothelial level might depend on some oxysterol profile present in the atheromasic lesion: the main cytotoxic oxysterols, i.e. 7-K, 7 β -OH, and 5 β ,6 β -epoxycholesterol (β -EPOX), are potent inducers of endolysosomal phospholipidosis, because they trigger the formation of multilamellar cytoplasmic structures, called myelin figures, containing high levels of phospholipids [62-64]. Phospholipid accumulation has been shown to be connected with oxidative stress and apoptosis induced by oxysterols, and could contribute to *in vivo* vascular injury [65]. Moreover, 7-K-induced phospholipidosis involves the phosphatidylinositol 3 kinase (PI3K)/Akt signaling pathway, and is partially reversed by vitamin E in U937 monocytes [66]. Of note, endolysosomal lipid storage induces strong pro-inflammatory and pro-atherogenic responses in macrophages [67].

Induction of other inflammatory mediators: phospholipase A₂ and cyclooxygenase-2

Treatment of rat aortic SMCs with 25-OH or 22R-OH causes accumulation of group IIA secretory phospholipase A₂ (PLA₂) and increases enzyme activity, with involvement of the LXR pathway [68]. Oxysterols are also able to activate the cytosolic PLA₂: in macrophages, activation of cytosolic PLA₂ by 25-OH increases arachidonic acid release [69] and is involved in the initiation of the apoptosis pathways triggered by 7-K [70]. In agreement with these observations, eicosanoid production has been found to be stimulated in bovine coronary artery ECs treated with 25-OH [62]. In contrast, in ECs, 7-K inhibits phosphorylation of cytosolic PLA₂ and arachidonic acid release, by altering a Ca²⁺-independent upstream step of the PI3K and ERK1/2 cascade [72].

The oxysterol TRIOL enhances cyclooxygenase-2 (COX-2) expression and synthesis, leading to prostaglandin E₂ (PGE₂) production in HUVECs. This effect requires the involvement of PI3K/Akt and endothelial nitric oxide synthase (NOS) and the activation of p38 MAPK and NF- κ B [73].

However, despite their marked pro-inflammatory effects, in certain experimental conditions, oxysterols have also been reported to exert anti-inflammatory effects. For instance, LPS-induced TNF- α secretion was significantly decreased when human macrophages were incubated with 7 β -OH, 25-OH and 27-OH before LPS challenge [74]. Moreover, exposure of human macrophages to chylomicron remnant-like particles loaded with 7 β -OH, but not similar particles loaded with 7-K, significantly reduced IL-6 and TNF- α secretion [75]. It is also reported that 22R-OH and 7-K inhibit protein expression of both inducible NOS and COX-2 in LPS-stimulated primary rat microglial cells [76].

LXR-dependent effects of oxysterols

LXR has been implicated in many biological processes, ranging from regulating lipid metabolism to inflammation and immunity [77-79]. Of note, LXR is involved in the pathogenesis of cardiovascular diseases, and LXR agonists prevent development of atherosclerosis, by modulating metabolic and inflammatory gene expression in rodent models. Indeed, activation of LXR leads to maintenance of cholesterol homeostasis through transcriptional activation, and to suppression of the inflammatory response through transrepression. Among the oxysterol family, 20S-hydroxycholesterol (20S-OH), 22R-OH, 24S-OH, 24S,25-epoxycholesterol (24S,25-EPOX), α -EPOX, 25-OH, and 27-OH are endogenous ligands of LXR, but 7-K and 7 β -OH are not [27,80-82]. However, among these LXR ligands, only 27-OH appears to be present in significant amounts in cholesterol-loaded human macrophages, cells that play a key role in atherogenesis [83].

Activation of both LXR isotypes (α and β) in cultured macrophages represses the expression of inflammatory mediators, such as inducible NOS, COX-2, IL-6 and IL-1 β , MCP-1 and MCP-3, and MMP-9, in response to inflammatory stimuli, and, at the same time, it regulates the expression of genes involved in lipid homeostasis. In this connection, LXR appears to play a major role in

translating lipid signaling into inflammatory responses [77,78,84]. Activation of LXR also inhibits inflammatory gene expression in the aortas of atherosclerotic mice [85]. However, it has recently been reported that agonists of the oxysterol LXR stimulate inflammatory pathways in ECs, leading to the up-regulation of adhesion molecules (e.g. ICAM, VCAM), chemokines (e.g. IL-8, IL-1 α), transcription factors (e.g. AP-1), and enzymes (e.g. COX-2), and to the down-regulation of endothelial NOS, through LXR-independent mechanisms [86].

Importantly, cross-talk between LXR and TLR signaling has been demonstrated in macrophages, as well as in aortic tissue. This cross-talk is mediated by the transcriptional factor IRF3, a specific effector of TLR3 and TLR4 that inhibits the transcriptional activity of LXR on its target promoters. Thus, activation of TLR3 and TLR4 can block the induction of LXR target genes by antagonizing LXR [77]. Conversely, activation of LXR by oxysterols or by synthetic agonists in macrophages inhibits TLR-inducible inflammatory genes, such as inducible NOS, IL-1 β , MCP-1, by interfering with NF- κ B signaling [78,87]. In this regard, the repression pathways mediated by LXR involve the nuclear receptor co-repressor. Nuclear receptor co-repressor-deficient macrophages exhibit derepression of a subset of genes that are normally activated by NF- κ B and AP-1 in response to pro-inflammatory signals [88,89].

Cholesteryl ester oxidation products

In the lipid moiety of LDLs, cholesterol is present both in the unesterified and in the esterified form, i.e. bound or not to fatty acids. The atherogenic properties of oxLDLs are also conferred by oxidized cholesteryl esters, in which oxidation involves either cholesterol itself or monounsaturated fatty acids and polyunsaturated fatty acids (PUFAs), producing several products, including cholesteryl ester hydroperoxides [90] and core-aldehydes [91]. Of note, at least in *in vitro* models, at more advanced stages of LDL oxidation, decomposition of the initially formed cholesteryl ester hydroperoxides may lead to the formation of oxysterols [92,93].

Induced formation of foam cells

Cholesteryl ester hydroperoxides are responsible for many of the biological activities of minimally oxLDLs, and they may be relevant to inflammatory activation of macrophages in atherosclerotic lesions [94]. Moreover, in differentiated human macrophages, it has been observed that oxidized derivatives of cholesteryl esters contribute to the formation of foam cells, by inducing scavenger receptor CD36 expression with involvement of PPAR α [95].

Although accumulation of cholesteryl oleate in the plasma has been reported to be a predictor of atherosclerosis in animal models, whereas cholesteryl esters of *n*-3 and *n*-6 PUFAs may provide athero-protection by reducing that accumulation [96], oxidized derivatives of cholesteryl ester containing *n*-6 PUFAs have been found in oxLDLs and in atherosclerotic lesions. In particular, high levels of 9-oxononanoyl cholesterol (9-ONC) and 5-oxovaleroyl cholesterol (5-OVC), respectively derived from linoleic acid and from arachidonic acid, have been found in oxLDLs and in atherosclerotic lesions [97-99]. These so-called core-aldehydes have been shown to stimulate adhesion molecule production by HUVECs, favoring blood monocytes and U937 cell adhesion [100]. Furthermore, these products can react covalently with amino groups of apoprotein B100, with consequent uptake of the modified oxLDLs by activated macrophages through scavenger receptors and foam cell formation [101].

Pro-fibrogenic effect

9-ONC has also been found to stimulate expression and synthesis of TGF β 1, in both J774.A1 murine macrophages and human U937 promonocytic cells, contributing to inflammation and fibrosclerosis in atherosclerotic plaques [102,103]. An increased membrane presentation of TGF β type II receptor has also been observed. The increased levels of the cytokine and of its

specific receptors in 9-ONC-treated cells appear to be mediated by the stimulation of ERK1/2 induced by the oxidized lipid. The core-aldehyde 9-ONC might thus sustain further vascular remodeling due to atherosclerosis, not simply by stimulating synthesis of the pro-fibrogenic cytokine TGF β 1 in vascular cells, but also and chiefly by enhancing the TGF β 1 autocrine loop [103].

Lipid peroxidation-derived free aldehydes

Lipids containing PUFAs are susceptible to free radical-initiated oxidation, and can participate in chain reactions that increase damage to biomolecules. The chain process of lipid peroxidation includes simultaneously generated lipid hydroperoxides and aldehydes of various chain lengths [104-108].

The enzymatic and non-enzymatic peroxidation of *n*-3 and *n*-6 PUFAs generates several reactive aldehyde species, which have been shown to exert regulatory roles as well as detrimental effects in various cell types and organs. These aldehydic end-products of lipid peroxidation are known to be among the molecules responsible for the pro-atherogenic effect of oxLDL.

Peroxidation of *n*-3 PUFAs, such as linolenic, eicosapentaenoic, and docosahexaenoic acids, generates the molecule 4-hydroxy-2-hexenal (HHE). Peroxidation of *n*-6 PUFAs, such as arachidonic and linoleic acids, as well as of their 15- and 13-lipoxygenase (15-LO, 13-LO) metabolites (namely 15-hydroperoxyeicosatetraenoic acid and 13-hydroperoxyoctadecadienoic acid) yields an aldehyde with remarkable biochemical effects, namely 4-hydroxy-2-nonenal (HNE). Another reactive peroxidation product is 4-hydroxy-2,6-dodecadienal (HDDE), which is derived from 12-hydroperoxyeicosatetraenoic acid, the 12-lipoxygenase metabolite of arachidonic acid. These aldehydes, and in particular HNE, have multiple pathophysiological effects, due to their high chemical reactivity and lipophilicity, and to their formation of covalent adducts with macromolecules (proteins, peptides, lipids, and nucleic acids). Indeed, they react with the thiol and

amino groups, especially of the amino acids cysteine, histidine and lysine. The progressive accumulation of these adducts alters normal cell functions, and may lead to cell death [104,109-111]. Nonetheless, at low and noncytotoxic concentrations, these molecules can act as second messengers in signal transduction in physiological and/or pathophysiological conditions [111-115].

The most representative unsaturated hydroxyalkenal in tissues and cells is HNE [116-118]. This aldehyde has been investigated in depth thanks to its contribution to the pathogenesis of major chronic human diseases, including atherosclerosis, and this molecule has been reported to possess both signaling and cytotoxic effects [112,116,119,120].

HNE-induced endothelial dysfunction and foam cell formation

HNE can directly impair the barrier function of the endothelium by increasing its permeability [115]: it may induce changes in cellular thiol redox status, thus perturbing cell signaling pathways and leading to endothelial barrier dysfunction. Following induction of ROS, HNE may affect EC permeability, modulating cell-cell adhesion by suppressing focal adhesion kinase (FAK) phosphorylation, which in turn affects focal adhesion, adherence, and tight junction proteins, as well as integrins, which are natural FAK receptors [121]. Modification of cytoskeletal proteins, including actin and microtubules, by HNE and by other unsaturated aldehydes, plays an important role in regulating cell-cell contacts and endothelial barrier function [121]. HNE may stimulate the adhesion of macrophages to the vascular endothelium in the early stages of atherosclerosis, as well as that of lymphocytes, pro-inflammatory immune cells [122]. This aldehyde may potentiate inflammation and immune responses by inducing COX-2 and in consequence prostaglandin production [123].

In addition, HNE covalently modifies LDLs by binding to lysine and histidine residues, leading to adduct formation [124]; this modification could make them available to scavenger receptors on macrophages, thus resulting in macrophage activation and foam cell formation, a

crucial event in the vascular inflammation that occurs in atherosclerotic lesions. HNE also promotes foam cell formation, by increasing synthesis of class A scavenger receptors at the transcriptional level, as well as increasing synthesis of the scavenger receptor CD36 [125]. HNE also induces induction of CD36 in vascular SMCs by enhancing nuclear accumulation of nuclear factor E2 related factor 2 (Nrf2), a second important transcription factor, alongside PPAR γ , in the induction of this scavenger receptor [126]. In this connection, it is also reported that nuclear translocation of Nrf2 requires activation of PKC [127]. After its translocation into the nucleus induced by HNE, Nrf2 transactivates the antioxidant responsive element (ARE). The HNE-induced Nrf2-ARE transcription pathway can up-regulate molecules such as thioredoxin (Trx), Trx reductase, and heme oxygenase-1 (HO-1), all of which are antioxidant proteins involved in the mechanisms of adapted cytoprotection against oxidative stress [128,129].

Pro-inflammatory effects of HNE

Again in regard to inflammation, HNE has been shown to increase MCP-1 release by J774.A1 macrophages, by activating PKC- β I and - β II. The aldehyde has a dual effect on these enzymes: their activity is increased if cells are incubated with relatively low concentrations of HNE, but progressively decreases if concentrations of the aldehyde are higher [130]. Strong up-regulation of the inflammatory and fibrogenic cytokine TGF β 1 in promonocytic and macrophagic cells challenged with pathophysiological concentrations of HNE has also been demonstrated [131-133].

It has been postulated that HNE-induced activation of NF- κ B mediates its pro-inflammatory and oxidative effects [134,135]. However, it is also reported that HNE may inactivate NF- κ B, by preventing proteolysis of the inhibitor of NF- κ B (I κ B) [136,137]. The dual effect of HNE on this redox transcriptional factor appears to depend on the type of cells used. For example, in THP-1 monocytes HNE inhibited NF- κ B activation induced by LPS and the subsequent TNF- α release, by

inhibiting phosphorylation and proteasome degradation of I κ B [136]. Further, in human aortic ECs, down-regulation of NF- κ B activity by HNE resulted in inhibition of the expression of various adhesion molecules induced by inflammatory stimuli [138]. In agreement with these findings, activation of NF- κ B induced by exposure of THP-1 cells to *Chlamydia pneumonia* was significantly reduced by HNE addition [139]. Addition of HNE to macrophages did not modify the constitutive extent of NF- κ B nuclear translocation [140]. In contrast, net stimulation of NF- κ B by HNE was demonstrated in vascular SMCs [141].

The different ways in which HNE can modulate the NF- κ B-signaling pathway may have a significant impact on atherosclerosis development. For instance, down-regulated expression of NF- κ B-dependent genes, as a consequence of HNE's inhibition of this nuclear factor, may interfere with the immune response and thus indirectly amplify and sustain inflammation and degenerative processes.

Conversely, marked activation of AP-1 nuclear binding by HNE has been reported. Activation of AP-1 has been demonstrated in murine and human macrophages [140], and in aortic SMCs [142]. HNE appears to up-regulate AP-1 DNA binding, mainly through activation of PKC- δ and, subsequently, through PKC-mediated activation of JNK [143].

Modulation of growth factor receptors by HNE

HNE interacts with epidermal growth factor receptor (EGFR) and platelet-derived growth factor receptor (PDGFR), which are transmembrane glycoproteins involved in various biological processes, as well as in the development of human diseases. In cultured human ECs, HNE was found to directly react with these membrane proteins, inducing formation of HNE-EGFR or HNE-PDGFR adducts [144]. However, HNE showed a dual effect on PDGFR- β , which regulates SMC migration and proliferation in the vascular wall. Short-term incubation of SMCs with low

concentrations of HNE induced autophosphorylation of PDGFR- β and activation of a signaling cascade involving the PI3K/Akt pathway and stimulating SMC proliferation [145]. Long-term incubation and high concentrations of HNE increased formation of HNE-PDGFR- β adducts and progressively inhibited PDGFR- β phosphorylation and SMC proliferation. The latter event may contribute to defective SMC proliferation, and decrease the stability of a vulnerable plaque [146].

Exposure to this aldehyde also resulted in increased proliferation of SMCs through MAPK activation. In rat aortic SMCs treated with HNE, strong but transient activation of ERK1/2 occurred, with induction of *c-fos* and *c-jun* protein synthesis, and increased AP-1 DNA binding activity; in addition, HNE induced PDGF-AA protein synthesis in rat aortic SMCs [142]. Again using SMCs, it was then shown that this aldehyde may activate not only ERK1/2 but also JNK and p38 MAPK [147]. Moreover, prolonged treatment of mouse aortic SMCs with a low concentration of HNE increased cell growth when young SMCs were used, but showed cytotoxic effects in aged SMCs. In young cells, the aldehyde induced strong activation of ERK and enhanced cyclin D1 expression [148]. Activation of PDGFR- β and ERK1/2 by HNE in human coronary SMCs also appeared to be the key mechanism in the production of MMP-1, which plays a role in SMC migration into the intima and in plaque instability [149].

HNE treatment of murine macrophages has been observed to enhance 5-LO, following its regulation at the transcriptional level by EGFR, through activation of the stimulating protein-1 (SP-1)/p38 and NF- κ B/ERK pathways [135]. Since HNE and 5-LO co-exist in macrophages in atherosclerotic lesions, it has been suggested that they may cooperate in modulating SMC migration, proliferation and atherosclerotic plaque instability. Indeed, the incubation of J774.A1 macrophagic cells with HNE induces production of 5-LO derivatives. The association of these products increases MMP-9 secretion via activation of ERK and p38 pathways [150]. The same research group also demonstrated a similar synergic action between HNE and 5-LO derivatives in vascular SMCs, in which marked induction of MMP-2 occurred, again following activation of the

ERK and p38 pathways [134,151]. Moreover, HNE has also been shown to stimulate MMP-2 production in the same type of cells, via mitochondrial ROS-mediated activation of the Akt/NF- κ B signaling pathways [134].

Pro-inflammatory effects of unsaturated aldehydes other than HNE

Notably, the finding that HNE and HDDE may act as endogenous ligands of PPAR δ in vascular ECs is particularly important in view of the remarkable metabolic and regulatory functions that this nuclear receptor mediates. For instance, selective PPAR δ ligands possess anti-inflammatory properties [152]; other reports have shown that PPAR δ also increases cholesterol export and represses inflammatory gene expression in macrophages [153], as well as augmenting fatty acid oxidation [154,155].

HHE, an end-product of *n*-3 PUFA peroxidation, is quite abundant but, due to its lower lipophilicity and lower reactivity, it is considered less physiologically active and less damaging than HNE or HDDE [104]. Further, HHE has properties in common with HNE, but there are important differences, particularly with regard to adduction targets and detoxification pathways [156]. It has been suggested that HHE may activate NF- κ B in vascular ECs, with a consequent increase in inducible NOS gene expression, which in turn leads to cell dysfunction due to excessive generation of nitric oxide radicals [157]. This aldehyde also appears to be responsible for EC apoptosis [158].

Another reactive unsaturated aldehyde is acrolein, which is generated from threonine by neutrophil myeloperoxidase at the site of inflammation, and which has been identified as both a product and an initiator of lipid peroxidation [159,160]. Elevated acrolein levels have been found in various diseases, including atherosclerosis, and it contributes to ROS generation, which in turn may lead to inflammation and cell dysfunction [161]. It has been demonstrated that acrolein induces COX-2 expression and PGE₂ production in HUVECs, through the activation of PKC, p38 MAPK,

and cAMP response element-binding protein (CREB). Moreover, this aldehyde inactivates Trx reductase, a primary antioxidant enzyme [161]. In this connection, acrolein, like HNE, reacts with the thiol groups of Trx1, a protein that regulates antioxidant functions in ECs. Modification of Trx1 by either aldehydes may potentiate monocyte adhesion to ECs during the early events of atherosclerosis [122]. Further, exposure to acrolein induces differentiated THP-1 macrophages to secrete MMP-9 via a mechanism that involves xanthine oxidase activation and increased ROS generation [162].

Oxidized phospholipids

Oxidized phospholipids (oxPLs) derive from oxidative modification of LDLs, but also from the membranes of cells undergoing apoptosis. Enzymatic or non-enzymatic oxidation of fatty acids linked to the *sn*-1 and *sn*-2 positions of glycerophospholipids leads to many different reaction products, depending on chain length and degree of unsaturation [111,163]. These products are implicated as modulators of inflammation, and increased levels of oxPLs are involved in the pathogenesis of various diseases, including atherosclerosis [164-166]. However, oxPLs play a dual role in modulating inflammation, since they may exhibit both pro-inflammatory and anti-inflammatory effects [16,165,167,168].

Primary role in stimulating adhesion of monocytes to the endothelial barrier

One major phospholipid present in minimally modified LDLs is 1-palmitoyl-2-arachidonoyl-*sn*-glycero-3-phosphorylcholine (PAPC), which generates a heterogeneous group of oxygenated full-length products, or compounds with truncated oxidized residues present at the *sn*-2 position (oxPAPC); these accumulate in atherosclerotic lesions [169,170]. The atherogenic potential of oxPLs, in particular of oxPAPC, has been demonstrated in cell culture studies, in terms of

enhanced binding of monocytes, but not of neutrophils, to oxPLs-stimulated ECs [170] and of concomitant induction of MCP-1 and IL-8 [171]: monocyte/EC adhesion is a hallmark of chronic inflammation. Regarding the mechanisms mediating adhesion of monocytes to ECs, oxPLs were not found to up-regulate ICAM, VCAM or E-selectin, but rather to induce MCP-1, connecting segment-1 and P-selectin. In this connection, oxPAPC have been shown to stimulate adhesion of monocytic cells through activation of β 1-integrin, which subsequently binds to a splice variant of fibronectin, containing the sequence known as connecting segment-1 [172]. The activation of β 1-integrin by oxPLs appears to be mediated by a cAMP-dependent R-Ras/PI3K pathway [173]. Of note, it has also been observed that oxPAPC induces monocyte adhesion to ECs by activating the PGE₂ receptor subtype 2, leading to increased cAMP levels and activation of PKA [174]. Another mechanism involves P-selectin, which is known to bind both monocytes and neutrophils to ECs. OxPLs up-regulates P-selectin in human aortic ECs [175] and in murine carotid arteries [176]. Up-regulation of P-selectin protein and P-selectin-dependent adhesion of leukocytes are also reported in ApoE^{-/-} mouse aortic segments [177]. Moreover, it has been suggested that oxPLs could stimulate formation of platelet-monocyte aggregates, an event that may play a role in the pathogenesis of vascular diseases, by enhancing P-selectin expression [178].

Increasing evidence also indicates that phosphatidylcholine (PC) hydroperoxide plays a role in atherosclerosis. In particular, it has been reported that treating THP-1 monocytic cells with PC hydroperoxide stimulates their adhesion to immobilized vascular EC adhesion molecules. In addition, THP-1 cell adhesion to ICAM-1, an effect exerted by this peroxide, was found to be dose-dependent [179] and to require activation of GTPases of the Rho subfamily named Rac (Ras-related C3 botulinum toxin substrate) [180].

Up-regulation of chemokines, inflammatory cytokines and growth factors

OxPLs, in particular oxPAPC, contribute to the recruitment of inflammatory cells in the atherosclerotic lesion, inducing not only adhesion molecules but also growth factors and cytokines in vascular ECs [181]. Moreover, induction by oxPLs of chemotactic activity in monocytes requires activation of MAPKs [182-184]. The induction of chemokines is regulated by genetic and/or epigenetic factors: in human aortic ECs derived from multiple heart transplant donors, the level of inflammatory gene induction by oxPLs differed. In particular, production of IL-8, but also of IL-6 and MCP-1, was increased in those cells through regulation of the unfolded protein response [185,186]. Further, immunohistochemical analysis of human atherosclerotic lesions indicated activation of the unfolded protein response in areas containing oxPLs [186]. Importantly, it has also been reported that up-regulation of IL-8 and MCP-1 expression induced by oxPLs is independent of the classical NF- κ B pathway, but that it involves activation of c-Src kinase. OxPLs bind to the TLR4 complex and lead to rapid and transient activation of c-Src kinase, which in turn induces phosphorylation of the transcription factor STAT3 [187]. Subsequently, STAT3 dimerizes, translocates to the nucleus and induces transcription of IL-8. In ECs, oxPAPC induces the synthesis of chemotactic factors, such as IL-8, involving c-Src kinase-dependent activation of JAK2, which leads to enhanced levels of STAT3 activity in inflammatory areas of atherosclerotic lesions [188]. Moreover, oxPAPC may modulate cytokine expression by binding to human macrophages via the platelet activating factor (PAF) receptor. Occupation of the PAF receptor by oxPLs stimulates intracellular calcium signaling, which modifies the transcription levels of highly pro-inflammatory cytokines, including IL-8 [189]. Another mechanism has also been proposed: oxPAPC might increase IL-8 and MCP-1 expression in ECs, involving activation of PPAR α [190,191].

The compound 1-palmitoyl-2-(5-oxovaleryl)-*sn*-glycero-phosphocholine (POVPC), an oxidatively fragmented component of oxPAPC, stimulates expression of IL-8, IL-1 β , and TNF- α in human monocyte-derived macrophages [189]. POVPC has also been found to activate cytosolic PLA₂ via the MAPK pathway in HUVECs, resulting in the release of free arachidonic acid [192],

which is used by LO to produce 12-hydroxyeicosatetraenoic acid that, in turn, contributes to stimulating monocyte binding to human aortic ECs [193].

In addition, hydroxy and oxo alkenal phospholipids, another form of oxPLs, may locate at the surface of oxLDLs and act as ligands of the scavenger receptor CD36 on macrophages, with consequent uptake of oxLDLs and foam cell formation [194].

In monocytes, oxPLs have also been found to stimulate VEGF that, in addition to its angiogenic growth factor activity, is another factor involved in leukocytes' migration [195]. In addition, up-regulation of VEGF induces expression of VCAM-1 and platelet endothelial cell adhesion molecule (PECAM-1) on the endothelium, and stimulates monocyte adhesion, leading to the formation of more inflamed atherosclerotic lesions in ApoE^{-/-} mice [196]. Again, in human aortic ECs, VEGF receptor 2 is reported to regulate ERK1/2-mediated activation of the transcription factor sterol regulatory element-binding protein 1 (SREBP-1), and the subsequent transcription of tissue factor, LDL receptor, and IL-8 [197]. Endothelial NOS has been reported to play a role in the activation of SREBP by oxPAPC [198].

OxPAPC may contribute to oxidative stress by inducing ROS generation, which in turn stimulates inflammatory gene expression. VEGF receptor 2 was found to mediate oxPAPC-induced recruitment of Rac1 to the NADPH oxidase-4 complex, resulting in strong ROS generation by ECs [199]. Another pathway used by oxPAPC to generate ROS includes uncoupled endothelial NOS and increased mitochondrial metabolism [200]. In parallel with an increased production of ROS, oxPAPC may stimulate the transcription of oxidative stress response genes, such as OKL38, a key regulator of important inflammatory and anti-inflammatory molecules [201]. Expression of OKL38 has been observed to be mediated by Nrf2 [202].

Anti-inflammatory effects

Despite their likely implication in inflammatory reactions, oxPLs are also thought to exert anti-inflammatory effects in leukocytes. For example, oxPLs, acting via PGE₂ receptor subtype 2, inhibit basal production of TNF- α and enhance production of the anti-inflammatory cytokine IL-10 in macrophages [203]. Inhibition of IL-12 synthesis, with a concomitant increase in IL-10 levels, has also been observed in primary human monocytes stimulated by TLR2 and TLR1 ligands [204]. Furthermore, oxPLs may exert anti-inflammatory effects, by inducing drug metabolism phase II genes, which mediate protection from oxidative stress. Low concentrations of oxPLs do not damage cells, but induce antioxidant enzymes, such as HO-1, known for its anti-inflammatory activity [205]. Induction of HO-1 expression by oxPAPC in HUVECs involves the phosphorylation of CREB, which is also dependent on MAPK pathways [206]. COX-2 is another enzyme involved in oxPL-mediated anti-inflammatory signaling. The expression of COX-2 appears to be modulated by oxPLs, in a cAMP-response-element-binding-protein-dependent and PPAR γ -dependent manner [207]. OxPLs may also increase NO production, with possible anti-inflammatory effects, including down-regulation of adhesion molecules and chemokines, as well as inhibition of leukocyte migration [198].

Modulation of vascular smooth muscle cell phenotype

Increasing experimental evidence suggests that oxPLs may play a role in phenotypic modulation of vascular SMCs. It has been found that oxPAPC and POVPC switch the phenotype of SMCs to an inflammatory state. Suppressed transcription of the SMC differentiation marker genes induces expression of pro-inflammatory genes, enhances the rate of cellular proliferation, and increases synthesis of extracellular matrix proteins; further, oxPLs can also suppress expression of smooth muscle actin and myosin heavy chain, and increase expression of MCP-1 and MCP-3 [208]. OxPL derivatives POVPC and 1-palmitoyl-2-glutaroyl-*sn*-glycero-3-phosphorylcholine (PGPC) have been shown, both *in vivo* and *in vitro*, to induce vascular SMC proliferation, by reducing

expression levels of connexin 43, another important factor in the pathogenesis of atherosclerosis [209]. In addition, POVPC may alter extracellular matrix production and stimulate vascular SMC migration [210]; it has also been shown that oxPLs stimulate expression of the metalloproteinase known as disintegrin, and that of metalloproteinase with thrombospondin motifs-1 (ADAMTS-1) [195]. These observations suggest a role of oxPLs in plaque destabilization.

Effects on endothelial cells

OxPLs, which accumulate in atherosclerotic lesions, may also exert intraplaque angiogenic effects, by stimulating production of VEGF, IL-8, and COX-2-derived prostaglandins that act in concert to induce the angiogenic switch in ECs [195]. A later study found that oxPLs induced up-regulation of VEGF by activating transcription factor 4 (ATF4)-dependent transcription [186]. Moreover, it has recently been reported that oxPLs regulate expression of ATF4 and VEGF in ECs, through a Nrf2-dependent mechanism [211].

OxPLs may have a dual effect on barrier function. Some *sn*-2 fragmented oxPLs, such as POVPC and PGPC, increase endothelial permeability even at low concentrations [212,213]. In contrast, both oxidized 1-palmitoyl-2-arachidonoyl-*sn*-glycero-3-phosphoserine (oxPAPS) and oxPAPC exhibit potent barrier-protective effects by attenuating the agonist-induced EC permeability triggered by transient activation of the Rho pathway [214]. Indeed, treatment of the endothelium with oxPAPC suppresses Rho-dependent induction of EC permeability, as stimulated by inflammatory molecules or other agonists, such as IL-6, thrombin, and LPS [212,214]. It has also been observed that, during protection of the endothelial barrier, oxPLs cause specific rearrangements of the cytoskeleton, also enlarging the adherens junctions and their colocalization with focal adhesion protein complexes [215].

Very recently, to elucidate the major pathways involved in oxPAPC action, a systems analysis of endothelial cell gene expression after exposure to these products was performed

[201,216]. This network analysis provided novel hypotheses about molecular interactions, as well as candidate molecular regulators of inflammation. In particular, it was found that oxPLs activate specific signaling pathways, and regulate a large number of genes involved in homeostasis of the cytoskeleton, junctional components, and tyrosine kinases, all cell elements that may contribute to the phenotypic and molecular changes observed in ECs treated with oxPAPC.

Other oxPLs derivatives: lysophospholipids

Hydrolysis of oxPLs by secretory PLA₂, or by lipoprotein associated-PLA₂, yields bioactive lipids that include oxidized fatty acids and lysophospholipids, such as lyso-phosphatidylcholine (lyso-PC) [217]. Lyso-PC, the precursor of lysophosphatidic acid, is found in atherosclerotic lesions and may in principle exert pro- or anti-atherogenic effects, depending on the arterial cell type and inflammation status. However, lyso-PC has been consistently shown to be associated with endothelial dysfunction in early coronary atherosclerosis in humans, thus supporting a role of these phospholipases in the pathogenesis of vascular inflammation and atheromasic lesions [218].

Various signaling effects relevant to atherosclerosis progression are reported to be exerted by lyso-PC. This lysophospholipid has been shown to: i) increase intracellular Ca²⁺, mainly by activating the PAF receptor, in macrophagic cells [219]; ii) modulate the MAPK pathway in macrophages [220]; iii) induce pro-inflammatory cytokines in circulating mononuclear cells, by activating the PAF receptor [221]; iv) activate G-protein-coupled receptor A, a molecule involved in immune cell migration and apoptosis, both in T lymphocytes and in macrophagic cells [222,223]. Further, lyso-PC has also been found to stimulate the release of pro-inflammatory cytokines, such as IL-8 and IL-6, and of growth factors, such as bFGF, in human coronary artery SMCs [224]. Even nanomolar concentrations of lyso-PC are able to stimulate cytokine expression and monocyte recruitment [225]. In addition, monocyte migration driven by lysophospholipids appears to

implicate a certain protein kinase D (PKD)-dependent signaling pathway [226] and to require non-selective cation channel activation, independent of G-protein and phospholipase C [227].

Arachidonic acid derivatives in atherosclerosis

Among the oxidized lipid products implicated in atherogenesis, there is a large group of potent bioactive lipids called eicosanoids (prostanoids, isprostanes, and leukotrienes) which derive from the oxidation of arachidonic acid, a component of membrane phospholipids released by PLA₂. Prostanoids and leukotrienes are formed enzymatically from arachidonic acid, by COX and LO, and the various sub-classes are generated by distinct enzymatic pathways. Isoprostanes are a class of prostaglandin-like products formed via free-radical-mediated oxidation of arachidonic acid, esterified in membrane phospholipids. All these lipid mediators are consistently observed in atherosclerotic lesions where, together with the oxLDL-derived oxidized lipids, they contribute to the inflammatory responses and to plaque progression [228-232].

Of note, LDL lipid oxidation products could regulate eicosanoid production, by modulating the enzymatic degradation of arachidonic acid via cyclooxygenases and lipoxygenases. In particular, oxLDL has been shown to downregulate inducible COX-2 in human macrophages exposed to LPS. This finding may support the hypothesis that transformation of macrophages into foam cells results in the attenuation of the inflammatory response, thus contributing to the progression of atherogenesis [233]. Inhibition of COX-2 by oxLDL might also promote thrombotic events, by disturbing the balance between platelet thromboxanes (TX) and PG [234]. In contrast, the aldehyde HNE is reported to induce gene expression of COX-2 [235]. It has also been hypothesized that 12-LO and 15-LO might directly contribute to LDL oxidation [236].

Prostanoids

Prostanoids, consisting of PG and TX, are produced from arachidonic acid by the sequential actions of PLA₂, COX-1 or COX-2, and the respective prostanoid synthases. They exert a variety of biological activities in the atheromasic lesion, by binding to specific G-protein-coupled receptors, known as the thromboxane receptor (TP) and the prostaglandin receptors (e.g. EP, IP), that are expressed in the target cells [237,238]. These receptors are highly expressed in ECs and SMCs, as well as in platelets and monocytes/macrophages. Of note, a significant increase in TP receptor expression and activation induces the expression of adhesion molecules, such as ICAM-1 and VCAM-1, in ECs [239,240], as well as inducing IL-1 β -dependent VCAM-1 expression in vascular SMCs [241] which facilitate the migration of monocytes to the vessel wall.

Prostanoids are known to promote the initiation and progression of atherosclerosis, not only via platelet activation, but also through leukocyte-endothelial adhesion and vasoconstriction. Of note, PGI₂ and TXA₂, the major prostanoids in cardiovascular diseases, have opposing actions: PGI₂ induces vascular relaxation, and potently inhibits platelet activation and vascular SMC proliferation, whereas TXA₂ is a potent vasoconstrictor, a strong platelet activator, and it also stimulates vascular SMC proliferation. Because of their opposed actions, the balance between these two bioactive lipids is known to be a critical factor causing a tendency to thrombosis. Moreover, it has been suggested that PGI₂ suppresses the development of vascular remodeling, due to its inhibitory actions on vascular SMC proliferation and on the platelet function [242] but also due to its regulatory role over endothelial progenitor cells, which are known to be the cells involved in reendothelialization, a process which limits vascular remodeling [243]. PGI₂ is also reported to play a role in the pro-angiogenic function of endothelial progenitor cells, consisting in proliferation of these cells and tube and capillary formation [244]. In contrast, platelets activated by TXA₂ serve as a source of growth factors, pro-inflammatory cytokines, and chemokines [245]. TXA₂ and its TP receptor are also critical to atherogenesis, promoting leukocyte recruitment and adhesion through enhancement of the transmembrane chemokine fractalkine (CX3CL1) [246].

The prostanoid 15-deoxy- $\Delta^{12,14}$ -prostaglandin J₂ (15d-PGJ₂) has also been shown to inhibit the cytokine-induced expression of several adhesion molecules, including E-selectin, ICAM-1 and VCAM-1. For example, 15d-PGJ₂ inhibits E-selectin expression via PPAR γ -dependent transcriptional induction of ATF3 [247]. Furthermore, 15d-PGJ₂ has a dual action on ICAM-1 in ECs: it directly induces ICAM-1 expression through an increase of AP-1 binding to its promoter, but it simultaneously blocks TNF- α -induced ICAM-1 expression through inhibition of NF- κ B DNA binding. Both these actions are PPAR γ -dependent [248]. TNF- α -induced VCAM-1 expression is also blocked by activation of the transmembrane PGI receptor, known as IP [249].

In the progression of atherosclerotic lesions, vascular SMCs migrate to the subendothelial space, where they proliferate and secrete extracellular matrix proteins. This process is partly mediated by endothelial production of PDGF, and 15d-PGJ₂ has been reported to inhibit PDGF synthesis in ECs, by decreasing the expression of the SP-1 transcription factor [250]. Of note, it has also been demonstrated that overexpression of COX-2 and of microsomal PGE synthase 1 play key roles in plaque instability, through enhanced MMP generation and activity [251]. In this connection, the prostaglandin PGE₂ might contribute to plaque destabilization, by inducing MMP-2 and MMP-9 activity in the macrophages present in the shoulder of atherosclerotic plaques [252,253]. In addition, PGE₂ could trigger neovascularization in the lesion, which enhances plaque growth and instability, by acting directly on ECs and inducing VEGF secretion through activation of ERK2 and JNK1 [254]. 15d-PGJ₂ might also have pro-angiogenic activity, by up-regulating VEGF expression with the involvement of PPAR γ [255]. PGE₂ might also exert pro- or anti-inflammatory effects, depending on its receptor subtypes (EP), the cell type, and the activation context. For example, it has been shown that EP3, a receptor for PGE, mediates PGE₂-exacerbated atherothrombosis [256] while the EP4 receptor inhibits production of chemokines by macrophages [257]. However, it is also reported that the EP4 receptor is involved in PGE₂-dependent MMP overexpression in human atherosclerotic plaques [258].

Isoprostanes

Several lines of evidence suggest that isoprostane generation may reflect oxidative stress and inflammation in experimental and human atherosclerosis [230,259]. Moreover, isoprostanes are useful biomarkers of cardiovascular diseases [260]. Some F₂-isoprostanes, in particular 8-iso-PGF_{2α}, have been demonstrated to have biological activities that may contribute to the progression of vascular damage [259].

Concerning the pro-inflammatory effects of F₂-isoprostanes, it has been observed that these arachidonic acid derivatives promote the formation of TXA₂, platelet activation, mitogenesis of vascular SMCs, proliferation of fibroblasts and ECs, and that they increase endothelin 1 expression in aortic ECs. All their biological activities involve activation of the TP receptor [261,262]. In particular, during promotion of platelet activation and adhesion, 8-iso-PGF_{2α}, induced activation of tyrosine kinase and of p38 MAPK [263] whereas in SMCs F₂-isoprostanes activated PKC, as well as Rho/Rho kinase and tyrosine kinases. In addition, activation of ERK1/2 by 8-iso-PGF_{2α} has been implicated in SMC proliferation while activation of p38 MAPK by the same isoprostane has been shown in the pro-adhesive phenotype of ECs [264,265]. Of note, 8-iso-PGF₂ has been observed to induce monocyte adhesion to ECs, although it was unable to induce the expression of E-selectin or VCAM-1: it induced monocyte adhesion via TP receptor and PKA/MAPK pathway activation [265]. Moreover, the isoprostane 8-iso-PGF_{2α} may induce formation of foam cells, by inducing scavenger receptor A type 1 expression, and it may stimulate oxLDL to induce MMP-9 and TIMP-1 gene expression in THP-1 cells [266].

Leukotrienes

Leukotrienes (LTs) are generated from arachidonic acid by the 5-, 12-, and 15-LO pathways [232]. Several studies have suggested the existence of a potential link between the LT signaling cascade and the progression of atherosclerosis. LTs exert pro-inflammatory effects, by interacting with specific LT receptors expressed in inflammatory cells within vascular wall [267].

Among LTs, LTB₄ produces increased leukocyte adhesion to the endothelium, followed by transendothelial migration. This inflammatory effect is mediated through the LTB₄-specific G-protein-coupled receptors, the BLT₁ and BLT₂ receptors [268]. In HUVECs, LTD₄ has been found to induce endothelial P-selectin expression through CysLT₂ receptor activation [269] and levels of various chemokines and of IL-8 were enhanced after stimulation of HUVECs with LTD₄ [270,271].

It has also been found that, within human atherosclerotic lesions, SMCs express receptors for LTB₄, mediating migration and proliferation of vascular SMCs [272]. Migration and proliferation of vascular SMCs appear to be modulated by β 3-integrin-associated signaling pathway activation, including FAK phosphorylation, mobilization of the actin cytoskeleton, association of FAK to PI3K, ERK phosphorylation, and NF- κ B activation [273]. Regarding the NF- κ B transcriptional factor, in cultured monocytic cells it has been found that LTB₄ phosphorylates MAPKs and stimulates NF- κ B activity, through the BLT₁ and BLT₂ membrane receptors. Activation of the transcriptional factor contributes to NF- κ B-dependent inflammatory responses [274].

It is also reported that 15-hydroxyeicosatetraenoic acid, generated by 15-lipoxygenase 2, plays a role in the regulation of vascular SMC migration and neointima formation, involving CREB-mediated IL-6 expression [275]. Another metabolite of 12- and 15-LO, 12-hydroxyeicosatetraenoic acid, can contribute to vascular SMC migration, by inducing MCP-1 and IL-6 expression, through Src tyrosine kinase signaling activation [276]. Further, 12-hydroxyeicosatetraenoic acid might activate PKC- α , which forms a complex with RhoA; this

complex is required for activation of NF- κ B and the subsequent stimulation of ICAM expression, which events favor monocyte adhesion to the endothelium [277].

Furthermore, LTB₄ has been shown to increase the intima/media thickness and expression of MMP-2 and MMP-9 in human arteries [278]. Again, production of LTB₄ and signaling through the BLT₁ receptor have recently been shown to be essential for MMP-2 secretion in vascular SMCs treated with HNE, leading to atherosclerotic plaque instability [279]. In this connection, the colocalization of 5-LO/LT generation with MMP in human carotid atherosclerotic lesions is reported [280].

Conclusions

All of the principal events in the multistep process of atherosclerosis, from initial EC damage, to atheroma development and progression, until its possible rupture, are now clearly recognized, and some insight into the relevant molecular mechanisms and cellular interplay has been achieved.

LDL lipid oxidation products, such as oxysterols, oxidized phospholipids, free and core-aldehydes, as well as lyso-PC, have all been shown to accumulate in atherosclerotic lesions, pointing to these lipids as important factors, not only in initiating but also in promoting the inflammatory process underlying atherosclerosis. Especially in the last decade, significant progress has been made in elucidating the role of these lipid oxidation products in cell signaling and gene transcription, related to (i) activation of ECs and SMCs, (ii) foam cell formation, extracellular matrix deposition and arterial wall remodeling, and (iii) modulation of the chronic inflammatory reactions that occur during development of the atherosclerotic lesion.

Figure 1 schematically depicts the putative involvement of oxidized lipids in the various steps of atherosclerosis. Current knowledge of oxLDL-mediated cell signaling definitely points to oxPLs as the pivotal molecules among LDL lipid oxidation products promoting endothelial cell

dysfunction. Its derivative compound lyso-PC also appears to be implicated in this early step of atherogenesis. Because of their ability to up-regulate MMP, oxPLs might also contribute to the progression of atherosclerotic lesions, especially those becoming vulnerable and unstable. However, a major role in the inflammation-driven formation of atheroma appears likely to be ascribable to oxysterols and to certain aldehydic end-products of PUFA oxidation, in particular to HNE. Both types of compounds have been shown to be crucially involved in foam cell formation, through net overexpression of the CD36 scavenger receptor; they are also very probably involved in up-regulating macrophage-SMC cross-talk, mainly through overexpression of the TGF β profibrogenic cytokine. In this connection, the demonstrated ability of the cholesteryl linoleate oxidation product 9-ONC to increase steady-state levels of both TGF β and TGF β receptors, in cells of the macrophage lineage, also appears interesting. Here, too, oxysterols, and less obviously free aldehydic products like HNE, are strong candidates for a key role in the process leading to the instability and rupture of the atherosclerotic plaque, because of their marked pro-apoptotic effect, and above all because of their demonstrated ability to up-regulate expression and activity of macrophage MMP-9, while constitutive levels of its specific inhibitors TIMP-1 and TIMP-2 remain unchanged.

Moreover, derivatives of arachidonic acid oxidation, besides affecting the initiation and progression of atherosclerotic lesions, play key roles in vascular tone modulation and vascular remodeling, mainly by regulating cell proliferation, platelet function and matrix deposition.

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Abbreviations: 5-OVC, 5-oxovaleroyl cholesterol; 7 α -OH, 7 α -hydroxycholesterol; 7 β -OH, 7 β -hydroxycholesterol; 7-K, 7-ketocholesterol; 9-ONC, 9-oxononanoyl cholesterol; 15d-PGJ₂, 15-deoxy- Δ 12,14-prostaglandin J₂; 20-OH, 20-hydroxycholesterol; 22-OH, 22-hydroxycholesterol; 24-OH, 24-hydroxycholesterol; 24S,25-EPOX, 24S,25-epoxycholesterol; 25-OH, 25-hydroxycholesterol; 27-OH, 27-hydroxycholesterol; α -EPOX, 5 α ,6 α -epoxycholesterol; β -EPOX, 5 β ,6 β -epoxycholesterol; AP-1, activator protein-1; ARE, antioxidant responsive element; ATF, activating transcription factor; bFGF, basic fibroblast growth factor; BLT, LTB receptor; COX, cyclooxygenase; CREB, cAMP response element-binding protein; ECs, endothelial cells; EGFR, epidermal growth factor receptor; EP, PGE receptor; ERK, extracellular signaling-regulated kinase; FAK, focal adhesion kinase; HDDE, 4-hydroxy-2,6-dodecadienal; HHE, 4-hydroxy-2-hexenal; HNE, 4-hydroxy-2-nonenal; HO-1, heme oxygenase-1; HUVECs, human umbilical vascular endothelial cells; ICAM, intercellular adhesion molecule; I κ B, inhibitor of NF- κ B; IL, interleukin; IP, PGI receptor; JAK, Janus kinase; JNK, c-Jun-N-terminal kinase; LDLs, low-density lipoprotein; LO, lipoxygenase; LPS, lipopolysaccharide; LT, leukotriene; LXR, liver X receptor; Lyso-PC, lyso-phosphatidylcholine; MAPKs, mitogen activated protein kinases; MCP-1, monocyte chemotactic protein-1; MIP-1 β , macrophage inflammatory protein-1 β ; MMP, metalloproteinase; NF- κ B, nuclear factor- κ B; NOS, nitric oxide synthase; Nrf2, nuclear factor E2 related factor 2; ox LDLs, oxidized low-density lipoproteins; oxPLs, oxidized phospholipids; PAF, platelet activity factor; PAPC, 1-palmitoyl-2-arachidonoyl-*sn*-glycero-3-phosphorylcholine; PAPS, 1-palmitoyl-2-arachidonoyl-*sn*-glycero-3-phosphoserine; PDGFR, platelet-derived growth factor receptor; PG, prostaglandin; PGPC, 1-palmitoyl-2-glutaroyl-*sn*-glycero-3-phosphorylcholine; PI3K, phosphatidylinositol 3-kinase; PK, protein kinase; PLA₂, phospholipase A₂; POVPC, 1-palmitoyl-2-(5-oxovaleryl)-*sn*-glycero-phosphocholine; PPAR, peroxisome proliferator-activated receptor; PUFAs, polyunsaturated fatty acids; RAC, Ras-related C3 botulinum toxin substrate; ROS, reactive

oxygen species; SMCs, smooth muscle cells; SP-1, stimulatory protein-1; SREBP-1, sterol regulatory element-binding protein 1; STAT, signal transducer and activator of transcription; TGF β 1, transforming growth factor β 1; TIMP, tissue inhibitor of metalloproteinase; TLR, Toll-like receptor; TNF- α , tumor necrosis factor- α ; TP, thromboxane receptor; TRIOL, cholestan-3 β ,5 α ,6 β -triol; Trx, thioredoxin; TX, thromboxane; VCAM, vascular cell adhesion molecule; VEGF, vascular endothelial growth factor.

REFERENCES

- [1] Witztum, J. L.; Steinberg, D. The oxidative modification hypothesis of atherosclerosis: does it hold for humans? *Trends Cardiovasc. Med.* **11**:93-102; 2001.
- [2] Tedgui, A.; Mallat, Z. Cytokines in atherosclerosis: pathogenic and regulatory pathways. *Physiol. Rev.* **86**:515-581; 2006.
- [3] Hansson, G. K. Inflammation, atherosclerosis, and coronary artery disease. *N. Engl. J. Med.* **352**:1685-1695; 2005.
- [4] Libby, P.; Ridker, P. M.; Hansson, G. K.; Leducq Transatlantic Network on Atherothrombosis. Inflammation in atherosclerosis: from pathophysiology to practice. *J. Am. Coll. Cardiol.* **54**:2129-2138; 2009.
- [5] Fougere, A.; Gayral, S.; Malet, N.; Briand-Mesange, F.; Breton-Douillon, M.; Laffargue, M. Phosphoinositide 3-kinases and their role in inflammation: potential clinical targets in atherosclerosis? *Clin. Sci. (Lond)*. **116**:791-804; 2009.
- [6] Libby, P.; Ridker, P. M.; Hansson, G. K. Progress and challenges in translating the biology of atherosclerosis. *Nature* **473**: 317-325; 2011.
- [7] Speyer, C. L.; Ward, P. A. Role of endothelial chemokines and their receptors during inflammation. *J. Invest. Surg.* **24**:18-27; 2011.

- [8] McLaren, J. E.; Michael, D. R.; Ashlin, T. G.; Ramji, D. P. Cytokines, macrophage lipid metabolism and foam cells: Implications for cardiovascular disease therapy. *Prog. Lipid Res.* **50**:331-347; 2011.
- [9] Steinberg, D. Atherogenesis in perspective: hypercholesterolemia and inflammation as partners in crime. *Nat. Med.* **8**:1211-1217; 2002.
- [10] Steinberg, D. Hypercholesterolemia and inflammation in atherogenesis: two sides of the same coin. *Mol. Nutr. Food Res.* **49**:995-998; 2005.
- [11] Cave, A. C.; Brewer, A. C.; Narayanapanicker, A.; Ray, R.; Grieve, D. J.; Walker, S.; Shah, A. M. NADPH oxidases in cardiovascular health and disease. *Antioxid. Redox Signal.* **8**:691-728; 2006.
- [12] Berliner, J. A.; Heinecke, J. W. The role of oxidized lipoproteins in atherogenesis. *Free Radic. Biol. Med.* **20**:707-727; 1996.
- [13] Badimon, L.; Storey, R. F.; Vilahur, G. Update on lipids, inflammation and atherothrombosis. *Thromb. Haemost.* **105 Suppl 1**:S34-42; 2011.
- [14] Leonarduzzi, G.; Arkan, M. C.; Başıağa, H.; Chiarpotto, E.; Sevanian, A.; Poli, G. Lipid oxidation products in cell signaling. *Free Radic. Biol. Med.* **28**:1370-1378; 2000.
- [15] Mazière, C.; Mazière, J. C. Activation of transcription factors and gene expression by oxidized low-density lipoprotein. *Free Radic. Biol. Med.* **46**:127-137; 2009.
- [16] Birukov, K. G. Oxidized lipids: the two faces of vascular inflammation. *Curr. Atheroscler. Rep.* **8**:223-231; 2006.
- [17] Schroepfer, G. J. Jr. Oxysterols: modulators of cholesterol metabolism and other processes. *Physiol. Rev.* **80**:361-554; 2000.
- [18] Leonarduzzi, G.; Sottero, B.; Poli, G. Oxidized products of cholesterol: dietary and metabolic origin, and proatherosclerotic effects (review). *J. Nutr. Biochem.* **13**:700-710; 2002.
- [19] Gill, S.; Chow, R.; Brown, A. J. Sterol regulators of cholesterol homeostasis and beyond: the oxysterol hypothesis revisited and revised. *Prog. Lipid Res.* **47**:391-404; 2008.

- [20] Smith, W. L.; Murphy, R. C. Oxidized lipids formed non-enzymatically by reactive oxygen species. *J. Biol. Chem.* **283**:15513-15514; 2008.
- [21] Brown, A. J.; Jessup, W. Oxysterols: Sources, cellular storage and metabolism, and new insights into their roles in cholesterol homeostasis. *Mol. Aspects Med.* **30**:111-122; 2009.
- [22] Poli, G.; Sottero, B.; Gargiulo, S.; Leonarduzzi, G. Cholesterol oxidation products in the vascular remodeling due to atherosclerosis. *Mol. Aspects Med.* **30**:180-189; 2009.
- [23] Sottero, B.; Gamba, P.; Gargiulo, S.; Leonarduzzi, G.; Poli, G. Cholesterol oxidation products and disease: an emerging topic of interest in medicinal chemistry. *Curr. Med. Chem.* **16**:685-705; 2009.
- [24] Otaegui-Arrazola, A.; Menéndez-Carreño, M.; Ansorena, D.; Astiasarán, I. Oxysterols: A world to explore. *Food Chem. Toxicol.* **48**:3289-3303; 2010.
- [25] Olkkonen, V. M.; Hynynen, R. Interactions of oxysterols with membranes and proteins. *Mol. Aspects Med.* **30**:123-133; 2009.
- [26] Shibata, N.; Glass, C. K. Macrophages, oxysterols and atherosclerosis. *Circ. J.* **74**:2045-2051; 2010.
- [27] Töröcsik, D.; Szanto, A.; Nagy, L. Oxysterol signaling links cholesterol metabolism and inflammation via the liver X receptor in macrophages. *Mol. Aspects Med.* **30**:134-152; 2009.
- [28] Leonarduzzi, G.; Gamba, P.; Sottero, B.; Kadl, A.; Robbesyn, F.; Calogero, R A.; Biasi, F.; Chiarpotto, E.; Leitinger, N.; Sevanian, A.; Poli, G. Oxysterol-induced up-regulation of MCP-1 expression and synthesis in macrophage cells. *Free Radic. Biol. Med.* **39**:1152-1161; 2005.
- [29] Gargiulo, S.; Sottero, B.; Gamba, P.; Chiarpotto, E.; Poli, G.; Leonarduzzi, G. Plaque oxysterols induce unbalanced up-regulation of matrix metalloproteinase-9 in macrophagic cells through redox-sensitive signaling pathways: Implications regarding the vulnerability of atherosclerotic lesions. *Free Radic. Biol. Med.* **51**:844-855; 2011.

- [30] Leonarduzzi, G.; Gargiulo, S.; Gamba, P.; Perrelli, M. G.; Castellano, I.; Sapino, A.; Sottero, B.; Poli, G. Molecular signaling operated by a diet-compatible mixture of oxysterols in up-regulating CD36 receptor in CD68 positive cells. *Mol. Nutr. Food Res.* **54 Suppl 1**:S31-41; 2010.
- [31] Prunet, C.; Montange, T.; Vélux, A.; Laubriet, A.; Rohmer, J. F.; Riedinger, J. M.; Athias, A.; Lemaire-Ewing, S.; Néel, D.; Petit, J. M.; Steinmetz, E.; Brenot, R.; Gambert, P.; Lizard, G. Multiplexed flow cytometric analyses of pro- and anti-inflammatory cytokines in the culture media of oxysterol-treated human monocytic cells and in the sera of atherosclerotic patients. *Cytometry A* **69**:359-373; 2006.
- [32] Muslin, A. J. MAPK signalling in cardiovascular health and disease: molecular mechanisms and therapeutic targets. *Clin. Sci. (Lond.)* **115**:203-218; 2008.
- [33] Liu, Y.; Hultén, L. M.; Wiklund, O. Macrophages isolated from human atherosclerotic plaques produce IL-8, and oxysterols may have a regulatory function for IL-8 production. *Arterioscler. Thromb. Vasc. Biol.* **17**:317-323; 1997.
- [34] Rydberg, E. K.; Salomonsson, L.; Hultén, L. M.; Norén, K.; Bondjers, G.; Wiklund, O.; Björnheden, T.; Ohlsson, B. G. Hypoxia increases 25-hydroxycholesterol-induced interleukin-8 protein secretion in human macrophages. *Atherosclerosis* **170**:245-252; 2003.
- [35] Lemaire-Ewing, S.; Prunet, C.; Montange, T.; Vélux, A.; Berthier, A.; Bessède, G.; Corcos, L.; Gambert, P.; Néel, D.; Lizard, G. Comparison of the cytotoxic, pro-oxidant and pro-inflammatory characteristics of different oxysterols. *Cell Biol. Toxicol.* **21**:97-114; 2005.
- [36] Erridge, C.; Webb, D. J.; Spickett, C. M. 25-Hydroxycholesterol, 7beta-hydroxycholesterol and 7-ketocholesterol upregulate interleukin-8 expression independently of Toll-like receptor 1, 2, 4 or 6 signalling in human macrophages. *Free Radic. Res.* **41**:260-266; 2007.
- [37] Lemaire-Ewing, S.; Berthier, A.; Royer, M. C.; Logette, E.; Corcos, L.; Bouchot, A.; Monier, S.; Prunet, C.; Raveneau, M.; Rébé, C.; Desrumaux, C.; Lizard, G.; Néel, D. 7beta-Hydroxycholesterol and 25-hydroxycholesterol-induced interleukin-8 secretion involves a calcium-

dependent activation of c-fos via the ERK1/2 signaling pathway in THP-1 cells: oxysterols-induced IL-8 secretion is calcium-dependent. *Cell Biol. Toxicol.* **25**:127-139; 2009.

[38] Bai, B.; Yamamoto, K.; Sato, H.; Sugiura, H.; Tanaka, T. Combined effect of 25-hydroxycholesterol and IL-1 β on IL-8 production in human colon carcinoma cell line (Caco-2). *Inflammation* **29**:141-146; 2005.

[39] Moreau, M.; Brocheriou, I.; Petit, L.; Ninio, E.; Chapman, M. J.; Rouis, M. Interleukin-8 mediates downregulation of tissue inhibitor of metalloproteinase-1 expression in cholesterol-loaded human macrophages: relevance to stability of atherosclerotic plaque. *Circulation* **99**:420-426; 1999.

[40] Palozza, P.; Simone, R.; Catalano, A.; Monego, G.; Barini, A.; Mele, M. C.; Parrone, N.; Trombino, S.; Picci, N.; Ranelletti, F. O. Lycopene prevention of oxysterol-induced proinflammatory cytokine cascade in human macrophages: inhibition of NF- κ B nuclear binding and increase in PPAR γ expression. *J. Nutr. Biochem.* **22**:259-268; 2011.

[41] Rosklint, T.; Ohlsson, B. G.; Wiklund, O.; Norén, K.; Hultén, L. M. Oxysterols induce interleukin-1 β production in human macrophages. *Eur. J. Clin. Invest.* **32**:35-42; 2002.

[42] Lizard, G.; Lemaire, S.; Monier, S.; Gueldry, S.; Néel, D.; Gambert, P. Induction of apoptosis and of interleukin-1 β secretion by 7 β -hydroxycholesterol and 7-ketocholesterol: partial inhibition by Bcl-2 overexpression. *FEBS Lett.* **419**:276-280; 1997.

[43] Lemaire, S.; Lizard, G.; Monier, S.; Miguët, C.; Gueldry, S.; Volot, F.; Gambert, P.; Néel, D. Different patterns of IL-1 β secretion, adhesion molecule expression and apoptosis induction in human endothelial cells treated with 7 α -, 7 β -hydroxycholesterol, or 7-ketocholesterol. *FEBS Lett.* **440**:434-439; 1998.

[44] Sung, S. C.; Kim, K.; Lee, K. A.; Choi, K. H.; Kim, S. M.; Son, Y. H.; Moon, Y. S.; Eo, S. K.; Rhim, B. Y. 7-Ketocholesterol upregulates interleukin-6 via mechanisms that are distinct from those of tumor necrosis factor- α , in vascular smooth muscle cells. *J. Vasc. Res.* **46**:36-44; 2009.

- [45] Ikeda, U.; Ito, T.; Shimada, K. Interleukin-6 and acute coronary syndrome. *Clin. Cardiol.* **24**:701-704; 2001.
- [46] Landis, M. S.; Patel, H. V.; Capone, J. P. Oxysterol activators of liver X receptor and 9-cis-retinoic acid promote sequential steps in the synthesis and secretion of tumor necrosis factor-alpha from human monocytes. *J. Biol. Chem.* **277**:4713-4721; 2002.
- [47] Feng, Y.; Schreiner, G. F.; Chakravarty, S.; Liu, D. Y.; Joly, A. H. Inhibition of the mitogen activated protein kinase, p38 alpha, prevents proinflammatory cytokine induction by human adherent mononuclear leukocytes in response to lipid loading. *Atherosclerosis* **158**:331-338; 2001.
- [48] Dushkin, M. I.; Khoshchenko, O. M.; Kudinova, E. N.; Schwartz, Y. Sh. Effects of hydroxysterols and atorvastatin on lipopolysaccharide-induced secretion of tumor necrosis factor and interleukin-10 by mouse macrophages. *Bull. Exp. Biol. Med.* **141**:233-235; 2006.
- [49] Dulak, J.; Józkowicz, A.; Dichtl, W.; Alber, H.; Schwarzacher, S. P.; Pachinger, O.; Weidinger, F. Vascular endothelial growth factor synthesis in vascular smooth muscle cells is enhanced by 7-ketocholesterol and lysophosphatidylcholine independently of their effect on nitric oxide generation. *Atherosclerosis* **159**:325-332; 2001.
- [50] Leonarduzzi, G.; Sevanian, A.; Sottero, B., Arkan, M. C.; Biasi, F.; Chiarotto, E.; Basaga, H.; Poli, G. Up-regulation of the fibrogenic cytokine TGF-beta1 by oxysterols: a mechanistic link between cholesterol and atherosclerosis. *FASEB J.* **15**:1619-1621; 2001.
- [51] Kraemer, R.; Pomerantz, K. B.; Joseph-Silverstein, J.; Hajjar, D. P. Induction of basic fibroblast growth factor mRNA and protein synthesis in smooth muscle cells by cholesteryl ester enrichment and 25-hydroxycholesterol. *J. Biol. Chem.* **268**:8040-8045; 1993.
- [52] Tamasawa, N.; Murakami, H.; Matsui, J.; Yamato, K.; JingZhi, G.; Imaizumi, T.; Fujimoto, K.; Yoshida, H.; Satoh, K.; Suda, T. An oxidized derivative of cholesterol increases the release of soluble vascular cell adhesion molecule-1 from human umbilical vein endothelial cells in culture. *Biochim. Biophys. Acta* **1531**:178-187; 2001.

- [53] Romeo, G.; Frangioni, J. V.; Kazlauskas, A. Profilin acts downstream of LDL to mediate diabetic endothelial cell dysfunction. *FASEB J.* **18**:725-727; 2004.
- [54] Naito, Y.; Shimozawa, M.; Manabe, H.; Nakabe, N.; Katada, K.; Kokura, S.; Yoshida, N.; Ichikawa, H.; Kon, T.; Yoshikawa, T. Azelnidipine, a new calcium channel blocker, inhibits endothelial inflammatory response by reducing intracellular levels of reactive oxygen species. *Eur. J. Pharmacol.* **546**:11-18; 2006.
- [55] Naito, Y.; Shimozawa, M.; Kuroda, M.; Nakabe, N.; Manabe, H.; Katada, K.; Kokura, S.; Ichikawa, H.; Yoshida, N.; Noguchi, N.; Yoshikawa, T. Tocotrienols reduce 25-hydroxycholesterol-induced monocyte-endothelial cell interaction by inhibiting the surface expression of adhesion molecules. *Atherosclerosis* **180**:19-25; 2005.
- [56] Romeo, G. R.; Kazlauskas, A. Oxysterol and diabetes activate STAT3 and control endothelial expression of profilin-1 via OSBP1. *J. Biol. Chem.* **283**:9595-9605; 2008.
- [57] Duran, M. J.; Pierre, S. V.; Lesnik, P.; Pieroni, G.; Bourdeaux, M.; Dignat-Georges, F.; Sampol, J.; Maixent, J. M. 7-ketocholesterol inhibits Na,K-ATPase activity by decreasing expression of its α 1-subunit and membrane fluidity in human endothelial cells. *Cell Mol. Biol. (Noisy-le-grand)*. **56 Suppl**:OL1434-OL441; 2010.
- [58] Leonarduzzi, G.; Gamba, P.; Gargiulo, S.; Sottero, B.; Kadl, A.; Biasi, F.; Chiarotto, E.; Leitinger, N.; Vendemiale, G.; Serviddio, G.; Poli, G. Oxidation as a crucial reaction for cholesterol to induce tissue degeneration: CD36 overexpression in human promonocytic cells treated with a biologically relevant oxysterol mixture. *Aging Cell* **7**:375-382; 2008.
- [59] Fuhrman, B.; Partoush, A.; Volkova, N.; Aviram, M. Ox-LDL induces monocyte-to-macrophage differentiation in vivo: Possible role for the macrophage colony stimulating factor receptor (M-CSF-R). *Atherosclerosis* **196**:598-607; 2008.
- [60] Miller, Y. I.; Choi, S. H.; Fang, L.; Harkewicz, R. Toll-like receptor-4 and lipoprotein accumulation in macrophages. *Trends Cardiovasc. Med.* **19**:227-232; 2009.

- [61] Kiechl, S.; Lorenz, E.; Reindl, M.; Wiedermann, C. J.; Oberhollenzer, F.; Bonora, E.; Willeit, J.; Schwartz, D. A. Toll-like receptor 4 polymorphisms and atherogenesis. *N. Engl. J. Med.* **347**:185-192; 2002.
- [62] Vejux, A.; Kahn, E.; Ménétrier, F.; Montange, T.; Lherminier, J.; Riedinger, J. M.; Lizard, G. Cytotoxic oxysterols induce caspase-independent myelin figure formation and caspase-dependent polar lipid accumulation. *Histochem. Cell. Biol.* **127**:609-624; 2007.
- [63] Vejux, A.; Malvitte, L.; Lizard, G. Side effects of oxysterols: cytotoxicity, oxidation, inflammation, and phospholipidosis. *Braz. J. Med. Biol. Res.* **41**:545-556; 2008.
- [64] Ors6, E.; Grandl, M.; Schmitz, G. Oxidized LDL-induced endolysosomal phospholipidosis and enzymatically modified LDL-induced foam cell formation determine specific lipid species modulation in human macrophages. *Chem. Phys. Lipids* **164**:479-487; 2011.
- [65] Miguet-Alfonsi, C.; Prunet, C.; Monier, S.; Bessède, G.; Lemaire-Ewing, S.; Berthier, A.; Ménétrier, F.; Néel, D.; Gambert, P.; Lizard, G. Analysis of oxidative processes and of myelin figures formation before and after the loss of mitochondrial transmembrane potential during 7 β -hydroxycholesterol and 7-ketocholesterol-induced apoptosis: comparison with various pro-apoptotic chemicals. *Biochem. Pharmacol.* **64**:527-541; 2002.
- [66] Vejux, A.; Guyot, S.; Montange, T.; Riedinger, J. M.; Kahn, E.; Lizard, G. Phospholipidosis and down-regulation of the PI3-K/PDK-1/Akt signalling pathway are vitamin E inhibitable events associated with 7-ketocholesterol-induced apoptosis. *J. Nutr. Biochem.* **20**:45-61; 2009.
- [67] Tabas, I. Macrophage death and defective inflammation resolution in atherosclerosis. *Nat. Rev. Immunol.* **10**:36-46; 2010.
- [68] Antonio, V.; Janvier, B.; Brouillet, A.; Andreani, M.; Raymondjean, M. Oxysterol and 9-cis-retinoic acid stimulate the group IIA secretory phospholipase A2 gene in rat smooth-muscle cells. *Biochem. J.* **376**(Pt 2):351-360; 2003.

- [69] Panini, S. R.; Yang, L.; Rusinol, A. E.; Sinensky, M. S.; Bonventre, J. V.; Leslie, C. C. Arachidonate metabolism and the signaling pathway of induction of apoptosis by oxidized LDL/oxysterol. *J. Lipid Res.* **42**:1678-1686; 2001.
- [70] Freeman, N. E.; Rusinol, A. E.; Linton, M.; Hachey, D. L.; Fazio, S.; Sinensky, M. S.; Thewke, D. Acyl-coenzyme A:cholesterol acyltransferase promotes oxidized LDL/oxysterol-induced apoptosis in macrophages. *J. Lipid Res.* **46**:1933-1943; 2005.
- [71] Wohlfeil, E. R.; Campbell, W. B. 25-Hydroxycholesterol enhances eicosanoid production in cultured bovine coronary artery endothelial cells by increasing prostaglandin G/H synthase-2. *Biochim. Biophys. Acta* **1345**:109-120; 1997.
- [72] Millanvove-Van Brussel, E.; Topal, G.; Brunet, A.; Do Pham, T.; Deckert, V.; Rendu, F.; David-Dufilho, M. Lysophosphatidylcholine and 7-oxocholesterol modulate Ca²⁺ signals and inhibit the phosphorylation of endothelial NO synthase and cytosolic phospholipase A2. *Biochem. J.* **380(Pt 2)**:533-539; 2004.
- [73] Liao, P. L.; Cheng, Y. W.; Li, C. H.; Lo, Y. L.; Kang, J. J. Cholesterol-3-beta, 5-alpha, 6-beta-triol induced PI(3)K-Akt-eNOS-dependent cyclooxygenase-2 expression in endothelial cells. *Toxicol. Lett.* **190**:172-178; 2009.
- [74] Englund, M. C.; Karlsson, A. L.; Wiklund, O.; Bondjers, G.; Ohlsson, B. G. 25-hydroxycholesterol induces lipopolysaccharide-tolerance and decreases a lipopolysaccharide-induced TNF-alpha secretion in macrophages. *Atherosclerosis* **158**:61-71; 2001.
- [75] Napolitano, M.; Bravo, E. Lipid metabolism and TNF-alpha secretion in response to dietary sterols in human monocyte derived macrophages. *Eur. J. Clin. Invest.* **35**:482-490; 2005.
- [76] Kim, O. S.; Lee, C. S.; Joe, E. H.; Jou, I. Oxidized low density lipoprotein suppresses lipopolysaccharide-induced inflammatory responses in microglia: oxidative stress acts through control of inflammation. *Biochem. Biophys. Res. Commun.* **342**:9-18; 2006.

- [77] Castrillo, A.; Joseph, S. B.; Vaidya, S. A.; Haberland, M.; Fogelman, A. M.; Cheng, G.; Tontonoz, P. Crosstalk between LXR and toll-like receptor signaling mediates bacterial and viral antagonism of cholesterol metabolism. *Mol. Cell* **12**:805-816; 2003.
- [78] Joseph, S. B.; Castrillo, A.; Laffitte, B. A.; Mangelsdorf, D. J., Tontonoz, P. Reciprocal regulation of inflammation and lipid metabolism by liver X receptors. *Nat. Med.* **9**:213-219; 2003.
- [79] Geyeregger, R.; Zeyda, M.; Stulnig, T. M. Liver X receptors in cardiovascular and metabolic disease. *Cell Mol. Life Sci.* **63**:524-539; 2006.
- [80] Edwards, P. A.; Kennedy, M.A.; Mak, P. A. LXRs; oxysterol-activated nuclear receptors that regulate genes controlling lipid homeostasis. *Vascul. Pharmacol.* **38**:249-256; 2002.
- [81] Khan, S. A.; Vanden Heuvel, J. P. Role of nuclear receptors in the regulation of gene expression by dietary fatty acids (review). *J. Nutr. Biochem.* **14**:554-567; 2003.
- [82] Berrodin, T. J.; Shen, Q.; Quinet, E. M.; Yudt, M. R. Freedman, L. P.; Nagpal, S. Identification of 5 α , 6 α -epoxycholesterol as a novel modulator of liver X receptor activity. *Mol. Pharmacol.* **78**:1046-1058; 2010.
- [83] Fu, X.; Menke, J. G.; Chen, Y.; Zhou, G.; MacNaul, K. L.; Wright, S. D; Sparrow, C. P.; Lund, E. G. 27-hydroxycholesterol is an endogenous ligand for liver X receptor in cholesterol-loaded cells. *J. Biol. Chem.* **276**:38378-38387; 2001.
- [84] Castrillo, A.; Joseph, S. B.; Marathe, C.; Mangelsdorf, D. J.; Tontonoz, P. Liver X receptor-dependent repression of matrix metalloproteinase-9 expression in macrophages. *J. Biol. Chem.* **278**:10443-10449; 2003.
- [85] Joseph, S. B.; McKilligin, E.; Pei, L.; Watson, M. A.; Collins, A. R.; Laffitte, B. A.; Chen, M.; Noh, G.; Goodman, J.; Hagger, G. N.; Tran, J.; Tippin, T. K.; Wang, X.; Lusis, A. J.; Hsueh, W. A.; Law, R. E.; Collins, J. L.; Willson, T. M.; Tontonoz, P. Synthetic LXR ligand inhibits the development of atherosclerosis in mice. *Proc. Natl. Acad. Sci. U. S. A.* **99**:7604-7609; 2002.
- [86] Morello, F.; Saglio, E.; Noghero, A.; Schiavone, D.; Williams, T. A.; Verhovez, A.; Bussolino, F.; Veglio, F.; Mulatero, P. LXR-activating oxysterols induce the expression of inflammatory

markers in endothelial cells through LXR-independent mechanisms. *Atherosclerosis* **207**:38-44; 2009.

[87] Huang, W.; Ghisletti, S.; Perissi, V.; Rosenfeld, M. G.; Glass, C. K. Transcriptional integration of TLR2 and TLR4 signaling at the NCoR derepression checkpoint. *Mol. Cell* **35**:48-57; 2009.

[88] Ogawa, S.; Lozach, J.; Jepsen, K.; Sawka-Verhelle, D.; Perissi, V.; Sasik, R.; Rose, D. W.; Johnson, R. S.; Rosenfeld, M. G.; Glass, C. K. A nuclear receptor corepressor transcriptional checkpoint controlling activator protein 1-dependent gene networks required for macrophage activation. *Proc. Natl. Acad. Sci. U. S. A.* **101**:14461-14466; 2004.

[89] Ghisletti, S.; Huang, W.; Ogawa, S.; Pascual, G.; Lin, M. E.; Willson, T. M.; Rosenfeld, M. G.; Glass, C. K. Parallel SUMOylation-dependent pathways mediate gene- and signal-specific transrepression by LXRs and PPARgamma. *Mol. Cell* **25**:57-70; 2007.

[90] Noguchi, N.; Nakano, K.; Aratani, Y.; Koyama, H.; Kodama, T.; Niki, E. Role of myeloperoxidase in the neutrophil-induced oxidation of low density lipoprotein as studied by myeloperoxidase-knockout mouse. *J. Biochem.* **127**:971-976; 2000.

[91] Ghosh, S.; Zhao, B.; Bie, J.; Song, J. Macrophage cholesteryl ester mobilization and atherosclerosis. *Vascul. Pharmacol.* **52**:1-10; 2010.

[92] Kritharides, L.; Jessup, W.; Gifford, J.; Dean, R. T. A method for defining the stages of low-density lipoprotein oxidation by the separation of cholesterol- and cholesteryl ester-oxidation products using HPLC. *Anal. Biochem.* **213**:79-89; 1993.

[93] Zarev, S.; Thérond, P.; Bonnefont-Rousselot, D.; Beaudoux, J. L.; Gardès-Albert, M.; Legrand, A. Major differences in oxysterol formation in human low density lipoproteins (LDLs) oxidized by OH/O_2^{\bullet} - free radicals or by copper. *FEBS Lett.* **451**:103-108; 1999.

[94] Harkewicz, R.; Hartvigsen, K.; Almazan, F.; Dennis, E. A.; Witztum, J. L.; Miller, Y. I. Cholesteryl ester hydroperoxides are biologically active components of minimally oxidized low density lipoprotein. *J. Biol. Chem.* **283**:10241-10251; 2008.

- [95] Jedidi, I.; Couturier, M.; Thérond, P.; Gardès-Albert, M.; Legrand, A.; Barouki, R.; Bonnefont-Rousselot, D.; Aggerbeck, M. Cholesteryl ester hydroperoxides increase macrophage CD36 gene expression via PPAR α . *Biochem. Biophys. Res. Commun.* **351**:733-738; 2006.
- [96] Degirolamo, C.; Shelness, G. S.; Rudel, L. L. LDL cholesteryl oleate as a predictor for atherosclerosis: evidence from human and animal studies on dietary fat. *J. Lipid Res.* **50 Suppl**:S434-S439; 2009.
- [97] Kamido, H.; Kuksis, A.; Marai, L.; Myher, J. J. Lipid ester-bound aldehydes among copper-catalyzed peroxidation products of human plasma lipoproteins. *J. Lipid Res.* **36**:1876-1886; 1995.
- [98] Hoppe, G.; Ravandi, A.; Herrera, D.; Kuksis, A.; Hoff, H. F. Oxidation products of cholesteryl linoleate are resistant to hydrolysis in macrophages, form complexes with proteins, and are present in human atherosclerotic lesions. *J. Lipid Res.* **38**:1347-1360; 1997.
- [99] Karten, B.; Boechzelt, H.; Abuja, P. M.; Mittelbach, M.; Sattler, W. Macrophage-enhanced formation of cholesteryl ester-core aldehydes during oxidation of low density lipoprotein. *J. Lipid Res.* **40**:1240-1253; 1999.
- [100] Huber, J.; Boechzelt, H.; Karten, B.; Surboeck, M.; Bochkov, V. N.; Binder, B. R.; Sattler, W.; Leitinger, N. Oxidized cholesteryl linoleates stimulate endothelial cells to bind monocytes via the extracellular signal-regulated kinase 1/2 pathway. *Arterioscler. Thromb. Vasc. Biol.* **22**:581-586; 2002.
- [101] Kawai, Y.; Saito, A.; Shibata, N.; Kobayashi, M.; Yamada, S.; Osawa, T.; Uchida, K. Covalent binding of oxidized cholesteryl esters to protein: implications for oxidative modification of low density lipoprotein and atherosclerosis. *J. Biol. Chem.* **278**:21040-21049; 2003.
- [102] Sottero, B.; Gamba, P.; Longhi, M.; Robbesyn, F.; Abuja, P. M.; Schaur, R. J.; Poli, G.; Leonarduzzi, G. Expression and synthesis of TGF β 1 is induced in macrophages by 9-oxononanoyl cholesterol, a major cholesteryl ester oxidation product. *Biofactors* **24**:209-216; 2005.
- [103] Gargiulo, S.; Gamba, P.; Sottero, B.; Biasi, F.; Chiarpotto, E.; Serviddio, G.; Vendemiale, G.; Poli, G.; Leonarduzzi, G. The core-aldehyde 9-oxononanoyl cholesterol increases the level of

transforming growth factor beta1-specific receptors on promonocytic U937 cell membranes. *Aging Cell* **8**:77-87; 2009.

[104] Esterbauer, H.; Schaur, R. J.; Zollner, H. Chemistry and biochemistry of 4-hydroxynonenal, malonaldehyde and related aldehydes. *Free Radic. Biol. Med.* **11**:81-128; 1991.

[105] Niki, E.; Yoshida, Y.; Saito, Y.; Noguchi, N. Lipid peroxidation: mechanisms, inhibition, and biological effects. *Biochem. Biophys. Res. Commun.* **338**:668-676; 2005.

[106] Niki, E. Lipid peroxidation: physiological levels and dual biological effects. *Free Radic. Biol. Med.* **47**:469-484; 2009.

[107] Schneider, C. An update on products and mechanisms of lipid peroxidation. *Mol. Nutr. Food Res.* **53**:315-321; 2009.

[108] Guéraud, F.; Atalay, M.; Bresgen, N.; Cipak, A.; Eckl, P. M.; Huc, L.; Jouanin, I.; Siems, W.; Uchida, K. Chemistry and biochemistry of lipid peroxidation products. *Free Radic. Res.* **44**:1098-1124; 2010.

[109] Petersen, D. R.; Doorn, J. A. Reactions of 4-hydroxynonenal with proteins and cellular targets. *Free Radic. Biol. Med.* **37**:937-945; 2004.

[110] Aldini, G.; Dalle-Donne, I.; Facino, R. M.; Milzani, A.; Carini, M. Intervention strategies to inhibit protein carbonylation by lipoxidation-derived reactive carbonyls. *Med. Res. Rev.* **27**:817-868; 2007.

[111] Catalá, A. Lipid peroxidation of membrane phospholipids generates hydroxy-alkenals and oxidized phospholipids active in physiological and/or pathological conditions. *Chem. Phys. Lipids* **157**:1-11; 2009.

[112] Poli, G.; Schaur, R. J.; Siems, W. G.; Leonarduzzi, G. 4-hydroxynonenal: a membrane lipid oxidation product of medicinal interest. *Med. Res. Rev.* **28**:569-631; 2008.

[113] Riahi, Y.; Cohen, G.; Shamni, O.; Sasson, S. Signaling and cytotoxic functions of 4-hydroxyalkenals. *Am. J. Physiol. Endocrinol. Metab.* **299**:879-886; 2010.

- [114] Forman, H. J. Reactive oxygen species and alpha,beta-unsaturated aldehydes as second messengers in signal transduction. *Ann. N.Y. Acad. Sci.* **1203**:35-44; 2010.
- [115] Usatyuk, P. V.; Natarajan, V. Hydroxyalkenals and oxidized phospholipids modulation of endothelial cytoskeleton, focal adhesion and adherens junction proteins in regulating endothelial barrier function. *Microvasc. Res.* In Press; 2011.
- [116] Poli, G.; Schaur, R. J. 4-Hydroxynonenal in the pathomechanisms of oxidative stress. *IUBMB Life* **50**:315-321; 2000.
- [117] Uchida, K. 4-Hydroxy-2-nonenal: a product and mediator of oxidative stress. *Prog. Lipid Res.* **42**:318-343; 2003.
- [118] Schneider, C.; Porter, N. A.; Brash, A. R. Routes to 4-hydroxynonenal: fundamental issues in the mechanisms of lipid peroxidation. *J. Biol. Chem.* **283**:15539-15543; 2008.
- [119] Dianzani, M. U. 4-hydroxynonenal from pathology to physiology. *Mol. Aspects Med.* **24**:263-272; 2003.
- [120] Leonarduzzi, G.; Robbesyn, F.; Poli, G. Signaling kinases modulated by 4-hydroxynonenal. *Free Radic. Biol. Med.* **37**:1694-1702; 2004.
- [121] Usatyuk, P. V.; Parinandi, N. L.; Natarajan, V. Redox regulation of 4-hydroxy-2-nonenal-mediated endothelial barrier dysfunction by focal adhesion, adherens, and tight junction proteins. *J. Biol. Chem.* **281**:35554-35566; 2006.
- [122] Go, Y. M.; Halvey, P. J.; Hansen, J. M.; Reed, M.; Pohl, J.; Jones, D. P. Reactive aldehyde modification of thioredoxin-1 activates early steps of inflammation and cell adhesion. *Am. J. Pathol.* **171**:1670-1681; 2007.
- [123] Uchida, K. A lipid-derived endogenous inducer of COX-2: a bridge between inflammation and oxidative stress. *Mol. Cells* **25**:347-351; 2008.
- [124] Annangudi, S. P.; Deng, Y.; Gu, X.; Zhang, W.; Crabb, J. W.; Salomon, R. G. Low-density lipoprotein has an enormous capacity to bind (E)-4-hydroxynon-2-enal (HNE): detection and

characterization of lysyl and histidyl adducts containing multiple molecules of HNE. *Chem. Res. Toxicol.* **21**:1384-1395; 2008.

[125] Yun, M. R.; Im, D. S.; Lee, S. J.; Woo, J. W.; Hong, K. W.; Bae, S. S.; Kim, C. D. 4-hydroxynonenal contributes to macrophage foam cell formation through increased expression of class A scavenger receptor at the level of translation. *Free Radic. Biol. Med.* **45**:177-183; 2008.

[126] Ishii, T.; Itoh, K.; Ruiz, E.; Leake, D. S.; Unoki, H.; Yamamoto, M.; Mann, G. E. Role of Nrf2 in the regulation of CD36 and stress protein expression in murine macrophages: activation by oxidatively modified LDL and 4-hydroxynonenal. *Circ. Res.* **94**:609-616; 2004.

[127] Numazawa, S.; Ishikawa, M.; Yoshida, A.; Tanaka, S.; Yoshida, T. Atypical protein kinase C mediates activation of NF-E2-related factor 2 in response to oxidative stress. *Am. J. Physiol. Cell. Physiol.* **285**:C334-C342; 2003.

[128] Siow, R. C.; Ishii, T.; Mann, G. E. Modulation of antioxidant gene expression by 4-hydroxynonenal: atheroprotective role of the Nrf2/ARE transcription pathway. *Redox Rep.* **12**:11-15; 2007.

[129] Tanito, M.; Agbaga, M. P.; Anderson, R. E. Upregulation of thioredoxin system via Nrf2-antioxidant responsive element pathway in adaptive-retinal neuroprotection in vivo and in vitro. *Free Radic. Biol. Med.* **42**:1838-1850; 2007.

[130] Nitti, M.; Domenicotti, C.; d'Abramo, C.; Assereto, S.; Cottalasso, D.; Melloni, E.; Poli, G.; Biasi, F.; Marinari, U. M.; Pronzato, M. A. Activation of PKC-beta isoforms mediates HNE-induced MCP-1 release by macrophages. *Biochem. Biophys. Res. Commun.* **294**:547-552; 2002.

[131] Leonarduzzi, G.; Scavazza, A.; Biasi, F.; Chiarpotto, E.; Camandola, S.; Vogel, S.; Dargel, R.; Poli, G. The lipid peroxidation end product 4-hydroxy-2,3-nonenal up-regulates transforming growth factor beta1 expression in the macrophage lineage: a link between oxidative injury and fibrosclerosis. *FASEB J.* **11**:851-857; 1997.

- [132] Chiarpotto, E.; Allasia, C.; Biasi, F.; Leonarduzzi, G.; Ghezzi, F.; Berta, G.; Bellomo, G., Waeg, G.; Poli, G. Down-modulation of nuclear localisation and pro-fibrogenic effect of 4-hydroxy-2,3-nonenal by thiol- and carbonyl-reagents. *Biochim. Biophys. Acta* **1584**:1-8; 2002.
- [133] Chiarpotto, E.; Castello, L.; Leonarduzzi, G.; Biasi, F.; Poli, G. Role of 4-hydroxy-2,3-nonenal in the pathogenesis of fibrosis. *Biofactors* **24**:229-236; 2005.
- [134] Lee, S. J.; Seo, K. W.; Yun, M. R.; Bae, S. S.; Lee, W. S.; Hong, K. W.; Kim, C. D. 4-Hydroxynonenal enhances MMP-2 production in vascular smooth muscle cells via mitochondrial ROS-mediated activation of the Akt/NF-kappaB signaling pathways. *Free Radic. Biol. Med.* **45**:1487-1492; 2008.
- [135] Lee, S. J.; Kim, C. E.; Seo, K. W.; Kim, C. D. HNE-induced 5-LO expression is regulated by NF- κ B/ERK and Sp1/p38 MAPK pathways via EGF receptor in murine macrophages. *Cardiovasc. Res.* **88**:352-359; 2010.
- [136] Page, S.; Fischer, C.; Baumgartner, B.; Haas, M.; Kreusel, U.; Loidl, G.; Hayn, M.; Ziegler-Heitbrock, H. W.; Neumeier, D.; Brand, K. 4-Hydroxynonenal prevents NF-kappaB activation and tumor necrosis factor expression by inhibiting IkappaB phosphorylation and subsequent proteolysis. *J. Biol. Chem.* **274**:11611-11618; 1999.
- [137] Hattori, Y.; Hattori, S.; Kasai, K. 4-hydroxynonenal prevents NO production in vascular smooth muscle cells by inhibiting nuclear factor-kappaB-dependent transcriptional activation of inducible NO synthase. *Arterioscler. Thromb. Vasc. Biol.* **21**:1179-1183; 2001.
- [138] Minekura, H.; Kumagai, T.; Kawamoto, Y.; Nara, F.; Uchida, K. 4-Hydroxy-2-nonenal is a powerful endogenous inhibitor of endothelial response. *Biochem. Biophys. Res. Commun.* **282**:557-561; 2001.
- [139] Donath, B.; Fischer, C.; Page, S.; Prebeck, S.; Jilg, N.; Weber, M.; da Costa, C.; Neumeier, D.; Miethke, T.; Brand, K. Chlamydia pneumoniae activates IKK/I kappa B-mediated signaling, which is inhibited by 4-HNE and following primary exposure. *Atherosclerosis* **165**:79-88; 2002.

- [140] Camandola, S.; Scavazza, A.; Leonarduzzi, G.; Biasi, F.; Chiarpotto, E.; Azzi, A.; Poli, G. Biogenic 4-hydroxy-2-nonenal activates transcription factor AP-1 but not NF-kappa B in cells of the macrophage lineage. *Biofactors* **6**:173-179; 1997.
- [141] Ruef, J.; Moser, M.; Bode, C.; Kübler, W.; Runge, M. S. 4-hydroxynonenal induces apoptosis, NF-kappaB-activation and formation of 8-isoprostane in vascular smooth muscle cells. *Basic. Res. Cardiol.* **96**:143-150; 2001.
- [142] Ruef, J.; Rao, G. N.; Li, F.; Bode, C.; Patterson, C.; Bhatnagar, A.; Runge, M. S. Induction of rat aortic smooth muscle cell growth by the lipid peroxidation product 4-hydroxy-2-nonenal. *Circulation* **97**:1071-1078; 1998.
- [143] Parola, M.; Robino, G.; Marra, F.; Pinzani, M.; Bellomo, G.; Leonarduzzi, G.; Chiarugi, P.; Camandola, S.; Poli, G.; Waeg, G.; Gentilini, P.; Dianzani, M. U. HNE interacts directly with JNK isoforms in human hepatic stellate cells. *J. Clin. Invest.* **102**:1942-1950; 1998.
- [144] Negre-Salvayre, A.; Vieira, O.; Escargueil-Blanc, I.; Salvayre, R. Oxidized LDL and 4-hydroxynonenal modulate tyrosine kinase receptor activity. *Mol. Aspects Med.* **24**:251-261; 2003.
- [145] Auge, N.; Garcia, V.; Maupas-Schwalm, F.; Levade, T.; Salvayre, R.; Negre-Salvayre, A. Oxidized LDL-induced smooth muscle cell proliferation involves the EGF receptor/PI-3 kinase/Akt and the sphingolipid signaling pathways. *Arterioscler. Thromb. Vasc. Biol.* **22**:1990-1995; 2002.
- [146] Vindis, C.; Escargueil-Blanc, I.; Uchida, K.; Elbaz, M.; Salvayre, R.; Negre-Salvayre, A. Lipid oxidation products and oxidized low-density lipoproteins impair platelet-derived growth factor receptor activity in smooth muscle cells: implication in atherosclerosis. *Redox Rep.* **12**:96-100; 2007.
- [147] Kakishita, H.; Hattori, Y. Vascular smooth muscle cell activation and growth by 4-hydroxynonenal. *Life Sci.* **69**:689-697; 2001.
- [148] Lee, T. J.; Lee, J. T.; Moon, S. K.; Kim, C. H.; Park, J. W.; Kwon, T. K. Age-related differential growth rate and response to 4-hydroxynonenal in mouse aortic smooth muscle cells. *Int. J. Mol. Med.* **17**:29-35; 2006.

- [149] Akiba, S.; Kumazawa, S.; Yamaguchi, H.; Hontani, N.; Matsumoto, T.; Ikeda, T.; Oka, M.; Sato, T. Acceleration of matrix metalloproteinase-1 production and activation of platelet-derived growth factor receptor beta in human coronary smooth muscle cells by oxidized LDL and 4-hydroxynonenal. *Biochim. Biophys. Acta* **1763**:797-804; 2006.
- [150] Lee, S. J.; Kim, C. E.; Yun, M. R.; Seo, K. W.; Park, H. M.; Yun, J. W.; Shin, H. K.; Bae, S. S.; Kim, C. D. 4-Hydroxynonenal enhances MMP-9 production in murine macrophages via 5-lipoxygenase-mediated activation of ERK and p38 MAPK. *Toxicol. Appl. Pharmacol.* **242**:191-198; 2010.
- [151] Seo, K. W.; Lee, S. J.; Kim, C. E.; Yun, M. R.; Park, H. M.; Yun, J. W.; Bae, S. S.; Kim, C. D. Participation of 5-lipoxygenase-derived LTB(4) in 4-hydroxynonenal-enhanced MMP-2 production in vascular smooth muscle cells. *Atherosclerosis* **208**:56-61; 2010.
- [152] Kilgore, K. S.; Billin, A. N. PPARbeta/delta ligands as modulators of the inflammatory response. *Curr. Opin. Investig. Drugs* **9**:463-469; 2008.
- [153] Fürnsinn, C.; Willson, T. M.; Brunmair, B. Peroxisome proliferator-activated receptor-delta, a regulator of oxidative capacity, fuel switching and cholesterol transport. *Diabetologia* **50**:8-17; 2007.
- [154] Cheng, L.; Ding, G.; Qin, Q.; Huang, Y.; Lewis, W.; He, N.; Evans, R. M.; Schneider, M. D.; Brako, F. A.; Xiao, Y.; Chen, Y. E.; Yang, Q. Cardiomyocyte-restricted peroxisome proliferator-activated receptor-delta deletion perturbs myocardial fatty acid oxidation and leads to cardiomyopathy. *Nat. Med.* **10**:1245-1250; 2004.
- [155] Risérus, U.; Sprecher, D.; Johnson, T.; Olson, E.; Hirschberg, S.; Liu, A.; Fang, Z.; Hegde, P.; Richards, D.; Sarov-Blat, L.; Strum, J. C.; Basu, S.; Cheeseman, J.; Fielding, B. A.; Humphreys, S. M.; Danoff, T.; Moore, N. R.; Murgatroyd, P.; O'Rahilly, S.; Sutton, P.; Willson, T.; Hassall, D.; Frayn, K. N.; Karpe, F. Activation of peroxisome proliferator-activated receptor (PPAR)delta promotes reversal of multiple metabolic abnormalities, reduces oxidative stress, and increases fatty acid oxidation in moderately obese men. *Diabetes* **57**:332-339; 2008.

- [156] Long, E. K.; Picklo, M. J. Sr. Trans-4-hydroxy-2-hexenal, a product of n-3 fatty acid peroxidation: make some room HNE... *Free Radic. Biol. Med.* **49**:1-8; 2010.
- [157] Lee, J. Y.; Je, J. H.; Jung, K. J.; Yu, B. P.; Chung, H. Y. Induction of endothelial iNOS by 4-hydroxyhexenal through NF-kappaB activation. *Free Radic. Biol. Med.* **37**:539-548; 2004.
- [158] Lee, J. Y.; Je, J. H.; Kim, D. H.; Chung, S. W.; Zou, Y.; Kim, N. D.; Ae Yoo, M.; Suck Baik, H.; Yu, B. P.; Chung, H. Y. Induction of endothelial apoptosis by 4-hydroxyhexenal. *Eur. J. Biochem.* **271**:1339-1347; 2004.
- [159] Anderson, M. M.; Hazen, S. L.; Hsu, F. F.; Heinecke, J. W. Human neutrophils employ the myeloperoxidase-hydrogen peroxide-chloride system to convert hydroxy-amino acids into glycolaldehyde, 2-hydroxypropanal, and acrolein. A mechanism for the generation of highly reactive alpha-hydroxy and alpha,beta-unsaturated aldehydes by phagocytes at sites of inflammation. *J. Clin. Invest.* **99**:424-432; 1997.
- [160] Stevens, J. F.; Maier, C. S. Acrolein: sources, metabolism, and biomolecular interactions relevant to human health and disease. *Mol. Nutr. Food Res.* **52**:7-25; 2008.
- [161] Park, Y. S.; Taniguchi, N. Acrolein induces inflammatory response underlying endothelial dysfunction: a risk factor for atherosclerosis. *Ann. N. Y. Acad. Sci.* **1126**:185-189; 2008.
- [162] O'Toole, T. E.; Zheng, Y. T.; Hellmann, J.; Conklin, D. J.; Barski, O.; Bhatnagar, A. Acrolein activates matrix metalloproteinases by increasing reactive oxygen species in macrophages. *Toxicol. Appl. Pharmacol.* **236**:194-201; 2009.
- [163] Fruhwirth, G. O.; Loidl, A.; Hermetter, A. Oxidized phospholipids: from molecular properties to disease. *Biochim. Biophys. Acta* **1772**:718-736; 2007.
- [164] Daigner, H. P.; Hermetter A. Oxidized phospholipids: emerging lipid mediators in pathophysiology. *Curr. Opin. Lipidol.* **19**:289-294; 2008.
- [165] Leitinger, N. The role of phospholipid oxidation products in inflammatory and autoimmune diseases: evidence from animal models and in humans. *Subcell. Biochem.* **49**:325-350; 2008.

- [166] Bochkov, V. N.; Oskolkova O. V.; Birukov, K. G.; Levonen, A. L.; Binder, C. J.; Stöckl, J. Generation and biological activities of oxidized phospholipids. *Antioxid. Redox Signal.* **12**:1009-1059; 2010.
- [167] Bochkov, V. N. Inflammatory profile of oxidized phospholipids. *Thromb. Haemost.* **97**:348-354; 2007.
- [168] Fu, P.; Birukov, K. G. Oxidized phospholipids in control of inflammation and endothelial barrier. *Transl. Res.* **153**:166-176; 2009.
- [169] Watson, A. D.; Leitinger, N.; Navab, M.; Faull, K. F.; Hörkkö, S.; Witztum, J. L.; Palinski, W.; Schwenke, D.; Salomon, R. G.; Sha, W.; Subbanagounder, G.; Fogelman, A. M.; Berliner, J. A. Structural identification by mass spectrometry of oxidized phospholipids in minimally oxidized low density lipoprotein that induce monocyte/endothelial interactions and evidence for their presence in vivo. *J. Biol. Chem.* **272**:13597-13607; 1997.
- [170] Subbanagounder, G.; Leitinger, N.; Schwenke, D. C.; Wong, J. W.; Lee, H.; Rizza, C.; Watson, A. D.; Faull, K. F.; Fogelman, A. M.; Berliner, J. A. Determinants of bioactivity of oxidized phospholipids. Specific oxidized fatty acyl groups at the sn-2 position. *Arterioscler. Thromb. Vasc. Biol.* **20**:2248-2254; 2000.
- [171] Berliner, J. A.; Subbanagounder, G.; Leitinger, N.; Watson, A. D.; Vora, D. Evidence for a role of phospholipid oxidation products in atherogenesis. *Trends Cardiovasc. Med.* **11**:142-147; 2001.
- [172] Leitinger, N.; Tyner, T. R.; Oslund, L.; Rizza, C.; Subbanagounder, G.; Lee, H.; Shih, P. T.; Mackman, N.; Tigyi, G.; Territo, M. C.; Berliner, J. A.; Vora, D. K. Structurally similar oxidized phospholipids differentially regulate endothelial binding of monocytes and neutrophils. *Proc. Natl. Acad. Sci. U. S. A.* **96**:12010-12015; 1999.
- [173] Cole, A. L.; Subbanagounder, G.; Mukhopadhyay, S.; Berliner, J. A.; Vora, D. K. Oxidized phospholipid-induced endothelial cell/monocyte interaction is mediated by a cAMP-dependent R-Ras/PI3-kinase pathway. *Arterioscler. Thromb. Vasc. Biol.* **23**:1384-1390; 2003.

- [174] Li, R.; Mouillesseaux, K. P.; Montoya, D.; Cruz, D.; Gharavi, N.; Dun, M.; Koroniak, L.; Berliner, J. A. Identification of prostaglandin E2 receptor subtype 2 as a receptor activated by OxPAPC. *Circ. Res.* **98**:642-650; 2006.
- [175] Vora, D. K.; Fang, Z. T.; Liva, S. M.; Tyner, T. R.; Parhami, F.; Watson, A. D.; Drake, T. A.; Territo, M. C.; Berliner, J. A. Induction of P-selectin by oxidized lipoproteins. Separate effects on synthesis and surface expression. *Circ. Res.* **80**:810-818; 1997.
- [176] Furnkranz, A.; Schober, A.; Bochkov, V. N.; Bashtrykov, P.; Kronke, G.; Kadl, A.; Binder, B. R.; Weber, C.; Leitinger, N. Oxidized phospholipids trigger atherogenic inflammation in murine arteries. *Arterioscler. Thromb. Vasc. Biol.* **25**:633-638; 2005.
- [177] Dever, G. J.; Benson, R.; Wainwright, C. L.; Kennedy, S.; Spickett, C. M. Phospholipid chlorohydrin induces leukocyte adhesion to ApoE^{-/-} mouse arteries via upregulation of P-selectin. *Free Radic. Biol. Med.* **44**:452-463; 2008.
- [178] Podrez, E. A.; Byzova, T. V.; Febbraio, M.; Salomon, R. G.; Ma, Y.; Valiyaveetil, M.; Poliakov, E.; Sun, M.; Finton, P. J.; Curtis, B. R.; Chen, J.; Zhang, R.; Silverstein, R. L.; Hazen, S. L. Platelet CD36 links hyperlipidemia, oxidant stress and a prothrombotic phenotype. *Nat. Med.* **13**:1086-1095; 2007.
- [179] Asai, A.; Okajima, F.; Nakagawa, K.; Ibusuki, D.; Tanimura, K.; Nakajima, Y.; Nagao, M.; Sudo, M.; Harada, T.; Miyazawa, T.; Oikawa, S. Phosphatidylcholine hydroperoxide-induced THP-1 cell adhesion to intracellular adhesion molecule-1. *J. Lipid Res.* **50**:957-965; 2009.
- [180] Asai, A.; Okajima, F.; Nakajima, Y.; Nagao, M.; Nakagawa, K.; Miyazawa, T.; Oikawa, S. Involvement of Rac GTPase activation in phosphatidylcholine hydroperoxide-induced THP-1 cell adhesion to ICAM-1. *Biochem. Biophys. Res. Commun.* **406**:273-277; 2011.
- [181] Berliner, J. A.; Watson, A. D. A role for oxidized phospholipids in atherosclerosis. *N. Engl. J. Med.* **353**:9-11. 2005.
- [182] Reddy, S.; Hama, S.; Grijalva, V.; Hassan, K.; Mottahedeh, R.; Hough, G.; Wadleigh, D. J.; Navab, M.; Fogelman, A. M. Mitogen-activated protein kinase phosphatase 1 activity is necessary

for oxidized phospholipids to induce monocyte chemotactic activity in human aortic endothelial cells. *J. Biol. Chem.* **276**:17030-17035; 2001.

[183] Bochkov, V. N.; Mechtcheriakova, D.; Lucerna, M.; Huber, J.; Malli, R.; Graier, W. F.; Hofer, E.; Binder, B. R.; Leitinger, N. Oxidized phospholipids stimulate tissue factor expression in human endothelial cells via activation of ERK/EGR-1 and Ca(++)/NFAT. *Blood* **99**:199-206; 2002.

[184] Birukov, K. G.; Leitinger, N.; Bochkov, V. N.; Garcia, J. G. Signal transduction pathways activated in human pulmonary endothelial cells by OxPAPC, a bioactive component of oxidized lipoproteins. *Microvasc. Res.* **67**:18-28; 2004.

[185] Gargalovic, P. S.; Imura, M.; Zhang, B.; Gharavi, N. M.; Clark, M. J.; Pagnon, J.; Yang, W. P.; He, A.; Truong, A.; Patel, S.; Nelson, S. F.; Horvath, S.; Berliner, J. A.; Kirchgessner, T. G.; Lusis, A. J. Identification of inflammatory gene modules based on variations of human endothelial cell responses to oxidized lipids. *Proc. Natl. Acad. Sci U. S. A.* **103**:12741-12746; 2006.

[186] Oskolkova, O. V.; Afonyushkin, T.; Leitner, A.; von Schlieffen, E.; Gargalovic, P. S.; Lusis, A. J.; Binder, B. R.; Bochkov, V. N. ATF4-dependent transcription is a key mechanism in VEGF up-regulation by oxidized phospholipids: critical role of oxidized sn-2 residues in activation of unfolded protein response. *Blood* **112**:330-339; 2008.

[187] Yeh, M.; Gharavi, N. M.; Choi, J.; Hsieh, X.; Reed, E.; Mouillesseaux, K. P.; Cole, A. L.; Reddy, S. T.; Berliner, J. A. Oxidized phospholipids increase interleukin 8 (IL-8) synthesis by activation of the c-src/signal transducers and activators of transcription (STAT)3 pathway. *J. Biol. Chem.* **279**:30175-30181; 2004.

[188] Gharavi, N. M.; Alva, J. A.; Mouillesseaux, K. P.; Lai, C.; Yeh, M.; Yeung, W.; Johnson, J.; Szeto, W. L.; Hong, L.; Fishbein, M.; Wei, L.; Pfeffer, L. M.; Berliner, J. A. Role of the Jak/STAT pathway in the regulation of interleukin-8 transcription by oxidized phospholipids in vitro and in atherosclerosis in vivo. *J. Biol. Chem.* **282**:31460-31468; 2007.

- [189] Pégrier, S.; Stengel, D.; Durand, H.; Croset, M.; Ninio, E. Oxidized phospholipid: POVPC binds to platelet-activating-factor receptor on human macrophages. Implications in atherosclerosis. *Atherosclerosis* **188**:433-443; 2006.
- [190] Lee, H.; Shi, W.; Tontonoz, P.; Wang, S.; Subbanagounder, G.; Hedrick, C. C.; Hama, S.; Borromeo, C.; Evans, R. M.; Berliner, J. A.; Nagy, L. Role for peroxisome proliferator-activated receptor alpha in oxidized phospholipid-induced synthesis of monocyte chemotactic protein-1 and interleukin-8 by endothelial cells. *Circ. Res.* **87**:516-521; 2000.
- [191] Subbanagounder, G.; Wong, J. W.; Lee, H.; Faull, K. F.; Miller, E.; Witztum, J. L.; Berliner, J. A. Epoxyisoprostane and epoxycyclopentenone phospholipids regulate monocyte chemotactic protein-1 and interleukin-8 synthesis. Formation of these oxidized phospholipids in response to interleukin-1beta. *J. Biol. Chem.* **277**:7271-7281; 2002.
- [192] Huber, J.; Fürnkranz, A.; Bochkov, V. N.; Patricia, M. K.; Lee, H.; Hedrick, C. C.; Berliner, J. A.; Binder, B. R.; Leitinger, N. Specific monocyte adhesion to endothelial cells induced by oxidized phospholipids involves activation of cPLA2 and lipoxygenase. *J. Lipid Res.* **47**:1054-1062; 2006.
- [193] Patricia, M. K.; Natarajan, R.; Dooley, A. N.; Hernandez, F.; Gu, J. L.; Berliner, J. A.; Rossi, J. J.; Nadler, J. L.; Meidell, R. S.; Hedrick, C. C. Adenoviral delivery of a leukocyte-type 12 lipoxygenase ribozyme inhibits effects of glucose and platelet-derived growth factor in vascular endothelial and smooth muscle cells. *Circ. Res.* **88**:659-665; 2001.
- [194] Podrez, E. A.; Poliakov, E.; Shen, Z.; Zhang, R.; Deng, Y.; Sun, M.; Finton, P. J.; Shan, L.; Gugiu, B.; Fox, P. L.; Hoff, H. F.; Salomon, R. G.; Hazen, S. L. Identification of a novel family of oxidized phospholipids that serve as ligands for the macrophage scavenger receptor CD36. *J. Biol. Chem.* **277**:38503-38516; 2002.
- [195] Bochkov, V. N.; Philippova, M.; Oskolkova, O.; Kadl, A.; Fürnkranz, A.; Karabeg, E.; Afonyushkin, T.; Gruber, F.; Breuss, J.; Minchenko, A.; Mechtcheriakova, D.; Hohensinner, P.; Rychli, K.; Wojta, J.; Resink, T.; Erne, P.; Binder, B. R.; Leitinger, N. Oxidized phospholipids

stimulate angiogenesis via autocrine mechanisms, implicating a novel role for lipid oxidation in the evolution of atherosclerotic lesions. *Circ. Res.* **99**:900-908; 2006.

[196] Lucerna, M.; Zerneck, A.; de Nooijer, R.; de Jager, S. C.; Bot, I.; van der Lans, C.; Kholova, I.; Liehn, E. A.; van Berkel, T. J.; Yla-Herttuala, S.; Weber, C.; Biessen, E. A. Vascular endothelial growth factor-A induces plaque expansion in ApoE knock-out mice by promoting de novo leukocyte recruitment. *Blood* **109**:122-129; 2007.

[197] Zimman, A.; Mouillesseaux, K. P.; Le, T.; Gharavi, N. M.; Ryvkin, A.; Graeber, T. G.; Chen, T. T.; Watson, A. D.; Berliner, J. A. Vascular endothelial growth factor receptor 2 plays a role in the activation of aortic endothelial cells by oxidized phospholipids. *Arterioscler. Thromb. Vasc. Biol.* **27**:332-338; 2007.

[198] Gharavi, N. M.; Baker, N. A.; Mouillesseaux, K. P.; Yeung, W.; Honda, H. M.; Hsieh, X.; Yeh, M.; Smart, E. J.; Berliner, J. A. Role of endothelial nitric oxide synthase in the regulation of SREBP activation by oxidized phospholipids. *Circ. Res.* **98**:768-776; 2006.

[199] Lee, S.; Gharavi, N. M.; Honda, H.; Chang, I.; Kim, B.; Jen, N.; Li, R.; Zimman, A.; Berliner, J. A. A role for NADPH oxidase 4 in the activation of vascular endothelial cells by oxidized phospholipids. *Free Radic. Biol. Med.* **47**:145-151; 2009.

[200] Landar, A.; Zmijewski, J. W.; Dickinson, D. A.; Le Goffe, C.; Johnson, M. S.; Milne, G. L.; Zanoni, G.; Vidari, G.; Morrow, J. D.; Darley-Usmar, V. M. Interaction of electrophilic lipid oxidation products with mitochondria in endothelial cells and formation of reactive oxygen species. *Am. J. Physiol. Heart Circ. Physiol.* **290**:1777-1787; 2006.

[201] Romanoski, C. E.; Che, N.; Yin, F.; Mai, N.; Pouladar, D.; Civelek, M.; Pan, C.; Lee, S.; Vakili, L.; Yang, W. P.; Kayne, P.; Mungrue, I. N.; Araujo, J. A.; Berliner, J. A.; Lusis, A. J. Network for activation of human endothelial cells by oxidized phospholipids: a critical role of heme oxygenase 1. *Circ. Res.* **109**:e27-41; 2011.

[202] Li, R.; Chen, W.; Yanes, R.; Lee, S.; Berliner, J. A. OKL38 is an oxidative stress response gene stimulated by oxidized phospholipids. *J. Lipid Res.* **48**:709-715; 2007.

- [203] Li, R.; Mouillesseaux, K. P.; Montoya, D.; Cruz, D.; Gharavi, N.; Dun, M.; Koroniak, L.; Berliner, J. A. Identification of prostaglandin E2 receptor subtype 2 as a receptor activated by OxPAPC. *Circ. Res.* **98**:642-650; 2006.
- [204] Cruz, D.; Watson, A. D.; Miller, C. S.; Montoya, D.; Ochoa, M. T.; Sieling, P. A.; Gutierrez, M. A.; Navab, M.; Reddy, S. T.; Witztum, J. L.; Fogelman, A. M.; Rea, T. H.; Eisenberg, D.; Berliner, J.; Modlin, R. L. Host-derived oxidized phospholipids and HDL regulate innate immunity in human leprosy. *J. Clin. Invest.* **118**:2917-2928; 2008.
- [205] Jyrkkänen, H. K.; Kansanen, E.; Inkala, M.; Kivelä, A. M.; Hurttila, H.; Heinonen, S. E.; Goldsteins, G.; Jauhiainen, S.; Tiainen, S.; Makkonen, H.; Oskolkova, O.; Afonyushkin, T.; Koistinaho, J.; Yamamoto, M.; Bochkov, V. N.; Ylä-Herttuala, S.; Levonen, A. L. Nrf2 regulates antioxidant gene expression evoked by oxidized phospholipids in endothelial cells and murine arteries in vivo. *Circ. Res.* **103**:1-9; 2008.
- [206] Krönke, G.; Bochkov, V. N.; Huber, J.; Gruber, F.; Blüml, S.; Fürnkranz, A.; Kadl, A.; Binder, B. R.; Leitinger, N. Oxidized phospholipids induce expression of human heme oxygenase-1 involving activation of cAMP-responsive element-binding protein. *J. Biol. Chem.* **278**:51006-51014; 2003.
- [207] Pontsler, A. V.; St Hilaire, A.; Marathe, G. K.; Zimmerman, G. A.; McIntyre, T. M. Cyclooxygenase-2 is induced in monocytes by peroxisome proliferator activated receptor gamma and oxidized alkyl phospholipids from oxidized low density lipoprotein. *J. Biol. Chem.* **277**:13029-13036; 2002.
- [208] Pidkovka, N. A.; Cherepanova, O. A.; Yoshida, T.; Alexander, M. R.; Deaton, R. A.; Thomas, J. A.; Leitinger, N.; Owens, G. K. Oxidized phospholipids induce phenotypic switching of vascular smooth muscle cells in vivo and in vitro. *Circ. Res.* **101**:792-801; 2007.
- [209] Johnstone, S. R.; Ross, J.; Rizzo, M. J.; Straub, A. C.; Lampe, P. D.; Leitinger, N.; Isakson, B. E. Oxidized phospholipid species promote in vivo differential cx43 phosphorylation and vascular smooth muscle cell proliferation. *Am. J. Pathol.* **175**:916-924; 2009.

- [210] Cherepanova, O. A.; Pidkovka, N. A.; Sarmiento, O. F.; Yoshida, T.; Gan, Q.; Adiguzel, E.; Bendeck, M. P.; Berliner, J.; Leitinger, N.; Owens, G. K. Oxidized phospholipids induce type VIII collagen expression and vascular smooth muscle cell migration. *Circ. Res.* **104**:609-618; 2009.
- [211] Afonyushkin, T.; Oskolkova, O. V.; Philippova, M.; Resink, T. J.; Erne, P.; Binder, B. R.; Bochkov, V. N. Oxidized phospholipids regulate expression of ATF4 and VEGF in endothelial cells via NRF2-dependent mechanism: novel point of convergence between electrophilic and unfolded protein stress pathways. *Arterioscler. Thromb. Vasc. Biol.* **30**:1007-1013; 2010.
- [212] Birukov, K. G.; Bochkov, V. N.; Birukova, A. A.; Kawkitinarong, K.; Rios, A.; Leitner, A.; Verin, A. D.; Bokoch, G. M.; Leitinger, N.; Garcia, J. G. Epoxycyclopentenone-containing oxidized phospholipids restore endothelial barrier function via Cdc42 and Rac. *Circ. Res.* **95**:892-901; 2004.
- [213] Qiao, J.; Huang, F.; Naikawadi, R. P.; Kim, K. S.; Said, T.; Lum, H. Lysophosphatidylcholine impairs endothelial barrier function through the G protein-coupled receptor GPR4. *Am. J. Physiol. Lung Cell Mol. Physiol.* **291**:91-101; 2006.
- [214] Birukova, A. A.; Fu, P.; Chatchavalvanich, S.; Burdette, D.; Oskolkova, O.; Bochkov, V. N.; Birukov, K. G. Polar head groups are important for barrier-protective effects of oxidized phospholipids on pulmonary endothelium. *Am. J. Physiol. Lung Cell Mol. Physiol.* **292**:924-935; 2007.
- [215] Birukova, A. A.; Malyukova, I.; Poroyko, V.; Birukov, K. G. Paxillin-beta-catenin interactions are involved in Rac/Cdc42-mediated endothelial barrier-protective response to oxidized phospholipids. *Am. J. Physiol. Lung Cell Mol. Physiol.* **293**:199-211; 2007.
- [216] Zimman, A.; Chen, S. S.; Komisopoulou, E.; Titz, B.; Martínez-Pinna, R.; Kafi, A.; Berliner, J. A.; Graeber, T. G. Activation of aortic endothelial cells by oxidized phospholipids: a phosphoproteomic analysis. *J. Proteome Res.* **9**:2812-2824; 2010.
- [217] Schmitz, G.; Ruebsaamen, K. Metabolism and atherogenic disease association of lysophosphatidylcholine. *Atherosclerosis* **208**:10-18; 2010.

- [218] Lavi, S.; McConnell, J. P.; Rihal, C. S.; Prasad, A.; Mathew, V.; Lerman, L. O.; Lerman, A. Local production of lipoprotein-associated phospholipase A2 and lysophosphatidylcholine in the coronary circulation: association with early coronary atherosclerosis and endothelial dysfunction in humans. *Circulation* **115**:2715-2721; 2007.
- [219] Ogita, T.; Tanaka, Y.; Nakaoka, T.; Matsuoka, R.; Kira, Y.; Nakamura, M.; Shimizu, T.; Fujita, T. Lysophosphatidylcholine transduces Ca²⁺ signaling via the platelet-activating factor receptor in macrophages. *Am. J. Physiol.* **272**:17-24; 1997.
- [220] Jing, Q.; Xin, S. M.; Zhang, W. B.; Wang, P.; Qin, Y. W.; Pei, G. Lysophosphatidylcholine activates p38 and p42/44 mitogen-activated protein kinases in monocytic THP-1 cells, but only p38 activation is involved in its stimulated chemotaxis. *Circ. Res.* **87**:52-59; 2000.
- [221] Huang, Y. H.; Schäfer-Elinder, L.; Wu, R.; Claesson, H. E.; Frostegård, J. Lysophosphatidylcholine (LPC) induces proinflammatory cytokines by a platelet-activating factor (PAF) receptor-dependent mechanism. *Clin. Exp. Immunol.* **116**:326-331; 1999.
- [222] Radu, C. G.; Yang, L. V.; Riedinger, M.; Au, M.; Witte, O. N. T cell chemotaxis to lysophosphatidylcholine through the G2A receptor. *Proc. Natl. Acad. Sci. U. S. A.* **101**:245-250; 2004.
- [223] Yang, L. V.; Radu, C. G.; Wang, L.; Riedinger, M.; Witte, O. N. Gi-independent macrophage chemotaxis to lysophosphatidylcholine via the immunoregulatory GPCR G2A. *Blood* **105**:1127-1134; 2005.
- [224] Aiyar, N.; Disa, J.; Ao, Z.; Ju, H.; Nerurkar, S.; Willette, R. N.; Macphee, C. H.; Johns, D. G.; Douglas, S. A. Lysophosphatidylcholine induces inflammatory activation of human coronary artery smooth muscle cells. *Mol. Cell. Biochem.* **295**:113-120; 2007.
- [225] Olofsson, K. E.; Andersson, L.; Nilsson, J.; Björkbacka, H. Nanomolar concentrations of lysophosphatidylcholine recruit monocytes and induce pro-inflammatory cytokine production in macrophages. *Biochem. Biophys. Res. Commun.* **370**:348-352; 2008.

- [226] Tan, M.; Hao, F.; Xu, X.; Chisolm, G. M.; Cui, M. Z. Lysophosphatidylcholine activates a novel PKD2-mediated signaling pathway that controls monocyte migration. *Arterioscler. Thromb. Vasc. Biol.* **29**:1376-1382; 2009.
- [227] Schilling, T.; Eder, C. Non-selective cation channel activity is required for lysophosphatidylcholine-induced monocyte migration. *J. Cell. Physiol.* **221**:325-334; 2009.
- [228] Praticò, D. Prostanoid and isoprostanoid pathways in atherogenesis. *Atherosclerosis* **201**:8-16; 2008.
- [229] Basu, S. Bioactive eicosanoids: role of prostaglandin F(2 α) and F₂-isoprostanes in inflammation and oxidative stress related pathology. *Mol. Cells* **30**:383-391; 2010.
- [230] Yuhki, K.; Kojima, F.; Kashiwagi, H.; Kawabe, J.; Fujino, T.; Narumiya, S.; Ushikubi, F. Roles of prostanoids in the pathogenesis of cardiovascular diseases: Novel insights from knockout mouse studies. *Pharmacol. Ther.* **129**:195-205; 2011.
- [231] Riccioni, G.; Bäck, M.; Capra, V. Leukotrienes and atherosclerosis. *Curr. Drug Targets* **11**:882-887; 2010.
- [232] Hersberger, M. Potential role of the lipoxygenase derived lipid mediators in atherosclerosis: leukotrienes, lipoxins and resolvins. *Clin. Chem. Lab. Med.* **48**:1063-1073; 2010.
- [233] Banfi, C.; Colli, S.; Eligini, S.; Mussoni, L.; Tremoli, E. Oxidized LDLs influence thrombotic response and cyclooxygenase 2. *Prostaglandins Leukot. Essent. Fatty Acids* **67**:169-173; 2002.
- [234] Linton, M. F.; Fazio, S. Cyclooxygenase-2 and inflammation in atherosclerosis. *Curr. Opin. Pharmacol.* **4**:116-123; 2004.
- [235] Uchida, K. A lipid-derived endogenous inducer of COX-2: a bridge between inflammation and oxidative stress. *Mol. Cells* **25**:347-351; 2008.
- [236] Wittwer, J.; Hersberger, M. The two faces of the 15-lipoxygenase in atherosclerosis. *Prostaglandins Leukot. Essent. Fatty Acids* **77**:67-77; 2007.

- [237] Alfranca, A.; Iñiguez, M. A.; Fresno, M.; Redondo, J. M. Prostanoid signal transduction and gene expression in the endothelium: role in cardiovascular diseases. *Cardiovasc. Res.* **70**:446-456; 2006.
- [238] Ricciotti, E.; FitzGerald, G. A. Prostaglandins and inflammation. *Arterioscler. Thromb. Vasc. Biol.* **31**:986-1000; 2011.
- [239] Ishizuka, T.; Suzuki, K.; Kawakami, M.; Hidaka, T.; Matsuki, Y.; Nakamura, H. Thromboxane A2 receptor blockade suppresses intercellular adhesion molecule-1 expression by stimulated vascular endothelial cells. *Eur. J. Pharmacol.* **312**:367-377; 1996.
- [240] Ishizuka, T.; Kawakami, M.; Hidaka, T.; Matsuki, Y.; Takamizawa, M.; Suzuki, K.; Kurita, A.; Nakamura, H. Stimulation with thromboxane A2 (TXA2) receptor agonist enhances ICAM-1, VCAM-1 or ELAM-1 expression by human vascular endothelial cells. *Clin. Exp. Immunol.* **112**:464-470; 1998.
- [241] Bayat, H.; Xu, S.; Pimentel, D.; Cohen, R. A.; Jiang, B. Activation of thromboxane receptor upregulates interleukin (IL)-1 β -induced VCAM-1 expression through JNK signaling. *Arterioscler. Thromb. Vasc. Biol.* **28**:127-134; 2008.
- [242] Cheng, Y.; Austin, S. C.; Rocca, B.; Koller, B. H.; Coffman, T. M.; Grosser, T.; Lawson, J. A.; FitzGerald, G. A. Role of prostacyclin in the cardiovascular response to thromboxane A2. *Science* **296**:539-541; 2002.
- [243] Kawabe, J.; Yuhki, K.; Okada, M.; Kanno, T.; Yamauchi, A.; Tashiro, N.; Sasaki, T.; Okumura, S.; Nakagawa, N.; Aburakawa, Y.; Takehara, N.; Fujino, T.; Hasebe, N.; Narumiya, S.; Ushikubi, F. Prostaglandin I2 promotes recruitment of endothelial progenitor cells and limits vascular remodeling. *Arterioscler. Thromb. Vasc. Biol.* **30**:464-470; 2010.
- [244] He, T.; Lu, T.; d'Uscio, L. V.; Lam, C. F.; Lee, H. C.; Katusic, Z. S. Angiogenic function of prostacyclin biosynthesis in human endothelial progenitor cells. *Circ. Res.* **103**:80-88; 2008.

- [245] Huo, Y.; Schober, A.; Forlow, S. B.; Smith, D. F.; Hyman, M. C.; Jung, S.; Littman, D. R.; Weber, C.; Ley, K. Circulating activated platelets exacerbate atherosclerosis in mice deficient in apolipoprotein E. *Nat. Med.* **9**:61-67; 2003.
- [246] Tole, S.; Durkan, A. M.; Huang, Y. W.; Liu, G.Y.; Leung, A.; Jones, L. L.; Taylor, J. A.; Robinson, L. A. Thromboxane prostanoid receptor stimulation induces shedding of the transmembrane chemokine CX3CL1 yet enhances CX3CL1-dependent leukocyte adhesion. *Am. J. Physiol. Cell. Physiol.* **298**:1469-1480; 2010.
- [247] Nawa, T.; Nawa, M. T.; Cai, Y.; Zhang, C.; Uchimura, I.; Narumi, S.; Numano, F.; Kitajima, S. Repression of TNF-alpha-induced E-selectin expression by PPAR activators: involvement of transcriptional repressor LRF-1/ATF3. *Biochem. Biophys. Res. Commun.* **275**:406-411; 2000.
- [248] Chen, N. G.; Han, X. Dual function of troglitazone in ICAM-1 gene expression in human vascular endothelium. *Biochem. Biophys. Res. Commun.* **282**:717-722; 2001.
- [249] Goya, K.; Otsuki, M.; Xu, X.; Kasayama, S. Effects of the prostaglandin I₂ analogue, beraprost sodium, on vascular cell adhesion molecule-1 expression in human vascular endothelial cells and circulating vascular cell adhesion molecule-1 level in patients with type 2 diabetes mellitus. *Metabolism* **52**:192-198; 2003.
- [250] Zhang, J.; Fu, M.; Zhao, L.; Chen, Y. E. 15-Deoxy-prostaglandin J(2) inhibits PDGF-A and -B chain expression in human vascular endothelial cells independent of PPAR gamma. *Biochem. Biophys. Res. Commun.* **298**:128-132; 2002.
- [251] Santovito, D.; Mezzetti, A.; Cipollone, F. Cyclooxygenase and prostaglandin synthases: roles in plaque stability and instability in humans. *Curr. Opin. Lipidol.* **20**:402-408; 2009.
- [252] Cipollone, F.; Prontera, C.; Pini, B.; Marini, M.; Fazia, M.; De Cesare, D.; Iezzi, A.; Uchino, S.; Boccoli, G.; Saba, V.; Chiarelli, F.; Cuccurullo, F.; Mezzetti, A. Overexpression of functionally coupled cyclooxygenase-2 and prostaglandin E synthase in symptomatic atherosclerotic plaques as a basis of prostaglandin E(2)-dependent plaque instability. *Circulation* **104**:921-927; 2001.

- [253] Cipollone, F.; Fazia, M.; Iezzi, A.; Pini, B.; Cuccurullo, C.; Zucchelli, M.; de Cesare, D.; Uchino, S.; Spigonardo, F.; De Luca, M.; Muraro, R.; Bei, R.; Bucci, M.; Cuccurullo, F.; Mezzetti, A. Blockade of the angiotensin II type 1 receptor stabilizes atherosclerotic plaques in humans by inhibiting prostaglandin E2-dependent matrix metalloproteinase activity. *Circulation* **109**:1482-1488; 2004.
- [254] Pai, R.; Szabo, I. L.; Soreghan, B. A.; Atay, S.; Kawanaka, H.; Tarnawski, A. S. PGE(2) stimulates VEGF expression in endothelial cells via ERK2/JNK1 signaling pathways. *Biochem. Biophys. Res. Commun.* **286**:923-928; 2001.
- [255] Inoue, M.; Itoh, H.; Tanaka, T.; Chun, T. H.; Doi, K.; Fukunaga, Y.; Sawada, N.; Yamshita, J.; Masatsugu, K.; Saito, T.; Sakaguchi, S.; Sone, M.; Yamahara, K.I.; Yurugi, T.; Nakao, K. Oxidized LDL regulates vascular endothelial growth factor expression in human macrophages and endothelial cells through activation of peroxisome proliferator-activated receptor-gamma. *Arterioscler. Thromb. Vasc. Biol.* **21**:560-566; 2001.
- [256] Gross, S.; Tilly, P.; Hentsch, D.; Vonesch, J. L.; Fabre, J. E. Vascular wall-produced prostaglandin E2 exacerbates arterial thrombosis and atherothrombosis through platelet EP3 receptors. *J. Exp. Med.* **204**:311-320; 2007.
- [257] Takayama, K.; García-Cardena, G.; Sukhova, G. K.; Comander, J.; Gimbrone, M. A. Jr.; Libby, P. Prostaglandin E2 suppresses chemokine production in human macrophages through the EP4 receptor. *J. Biol. Chem.* **277**:44147-44154; 2002.
- [258] Cipollone, F.; Cicolini, G.; Bucci, M. Cyclooxygenase and prostaglandin synthases in atherosclerosis: recent insights and future perspectives. *Pharmacol. Ther.* **118**:161-180; 2008.
- [259] Minuz, P.; Fava, C.; Lechi, A. Lipid peroxidation, isoprostanes and vascular damage. *Pharmacol. Rep.* **58**:57-68; 2006.
- [260] Davies, S. S.; Roberts, L. J. 2nd. F₂-isoprostanes as an indicator and risk factor for coronary heart disease. *Free Radic. Biol. Med.* **50**:559-566; 2011.

- [261] Tang, M.; Cyrus, T.; Yao, Y.; Vocun, L.; Praticò, D. Involvement of thromboxane receptor in the proatherogenic affect of isoprostone $F_{2\alpha}$ -III. Evidence from apolipoprotein E- and LDL receptor-deficient mice. *Circulation* **112**:2867-2874; 2005.
- [262] Comporti, M.; Signorini, C.; Arezzini, B.; Vecchio, D.; Monaco, B.; Gardi, C. F2-isoprostanes are not just markers of oxidative stress. *Free Radic. Biol. Med.* **44**:247-256; 2008.
- [263] Minuz, P.; Gaino, S.; Zuliani, V.; Tommasoli, R. M.; Benati, D.; Ortolani, R.; Zancanaro, C.; Berton, G.; Santonastaso, C. L. Functional role of p38 mitogen activated protein kinase in platelet activation induced by a thromboxane A_2 analogue and by 8-iso-prostaglandin F_2 . *Thromb. Haemost.* **87**:888-898; 2002.
- [264] Habib, A.; Badr, K. F. Molecular pharmacology of isoprostanes in vascular smooth muscle cells. *Chem. Phys. Lipids* **128**:69-73; 2004.
- [265] Leitinger, N.; Huber, J.; Rizza, C.; Mechtcheriakova, D.; Bochkov, V.; Koshelnick, Y.; Berliner, J. A.; Binder, B. R. The isoprostone 8-iso-PG $_{2\alpha}$ stimulates endothelial cells to bind monocytes: differences from thromboxane-mediated endothelial activation. *FASEB J.* **15**:1254-1256; 2001.
- [266] Scholz, H.; Aukrust, P.; Damås, J. K.; Tonstad, S.; Sagen, E. L.; Kolset, S. O.; Hall, C.; Yndestad, A.; Halvorsen, B. 8-Isoprostone increases scavenger receptor A and matrix metalloproteinase activity in THP-1 macrophages, resulting in long-lived foam cells. *Eur. J. Clin. Invest.* **34**:451-458; 2004.
- [267] Bäck, M. Leukotriene signaling in atherosclerosis and ischemia. *Cardiovasc. Drugs Ther.* **23**:41-48; 2009.
- [268] Funk, C. D. Prostaglandins and leukotrienes: advances in eicosanoid biology. *Science* **294**:1871-1875; 2001.
- [269] Pedersen, K. E.; Bochner, B. S.; Undem, B. J. Cysteinyl leukotrienes induce P-selectin expression in human endothelial cells via a non-CysLT1 receptor-mediated mechanism. *J. Pharmacol. Exp. Ther.* **281**:655-662; 1997.

- [270] Zhao, L.; Moos, M. P.; Gräbner, R.; Pédrone, F.; Fan, J.; Kaiser, B.; John, N.; Schmidt, S.; Spanbroek, R.; Lötzer, K.; Huang, L.; Cui, J.; Rader, D. J.; Evans, J. F.; Habenicht, A. J.; Funk, C. D. The 5-lipoxygenase pathway promotes pathogenesis of hyperlipidemia-dependent aortic aneurysm. *Nat. Med.* **10**:966-973; 2004.
- [271] Uzonyi, B.; Lötzer, K.; Jahn, S.; Kramer, C.; Hildner, M.; Bretschneider, E.; Radke, D.; Beer, M.; Vollandt, R.; Evans, J. F.; Funk, C. D.; Habenicht, A. J. Cysteinyl leukotriene 2 receptor and protease-activated receptor 1 activate strongly correlated early genes in human endothelial cells. *Proc. Natl. Acad. Sci. U.S.A.* **103**:6326-6331; 2006.
- [272] Bäck, M.; Bu, D. X.; Bränstrom, R.; Sheikine, Y.; Yan, Z. Q.; Hansson, G. K. Leukotriene B4 signaling through NF-kappaB-dependent BLT1 receptors on vascular smooth muscle cells in atherosclerosis and intimal hyperplasia. *Proc. Natl. Acad. Sci. U.S.A.* **102**:17501-17506; 2005.
- [273] Moraes, J.; Assreuy, J.; Canetti, C.; Barja-Fidalgo, C. Leukotriene B4 mediates vascular smooth muscle cell migration through $\alpha\text{v}\beta 3$ integrin transactivation. *Atherosclerosis* **212**:406-413; 2010.
- [274] Sánchez-Galán, E.; Gómez-Hernández, A.; Vidal, C.; Martín-Ventura, J. L.; Blanco-Colio, L. M.; Muñoz-García, B.; Ortega, L.; Egido, J.; Tuñón, J. Leukotriene B4 enhances the activity of nuclear factor-kappaB pathway through BLT1 and BLT2 receptors in atherosclerosis. *Cardiovasc. Res.* **81**:216-225; 2009.
- [275] Chava, K. R.; Karpurapu, M.; Wang, D.; Bhanoori, M.; Kundumani-Sridharan, V.; Zhang, Q.; Ichiki, T.; Glasgow, W. C.; Rao, G. N. CREB-mediated IL-6 expression is required for 15(S)-hydroxyeicosatetraenoic acid-induced vascular smooth muscle cell migration. *Arterioscler. Thromb. Vasc. Biol.* **29**:809-815; 2009.
- [276] Reddy, M. A.; Sahar, S.; Villeneuve, L. M.; Lanting, L.; Natarajan, R. Role of Src tyrosine kinase in the atherogenic effects of the 12/15-lipoxygenase pathway in vascular smooth muscle cells. *Arterioscler. Thromb. Vasc. Biol.* **29**:387-393; 2009.

- [277] Bolick, D. T.; Orr, A. W.; Whetzel, A.; Srinivasan, S.; Hatley, M. E.; Schwartz, M. A.; Hedrick, C. C. 12/15-lipoxygenase regulates intercellular adhesion molecule-1 expression and monocyte adhesion to endothelium through activation of RhoA and nuclear factor-kappaB. *Arterioscler. Thromb. Vasc. Biol.* **25**:2301-2307; 2005.
- [278] Hlawaty, H.; Jacob, M. P.; Louedec, L.; Letourneur, D.; Brink, C.; Michel, J. B.; Feldman, L.; Bäck, M. Leukotriene receptor antagonism and the prevention of extracellular matrix degradation during atherosclerosis and in-stent stenosis. *Arterioscler. Thromb. Vasc. Biol.* **29**:518-524; 2009.
- [279] Seo, K. W.; Lee, S. J.; Kim, C. E.; Yun, M. R.; Park, H. M.; Yun, J. W.; Bae, S. S.; Kim, C. D. Participation of 5-lipoxygenase-derived LTB(4) in 4-hydroxynonenal-enhanced MMP-2 production in vascular smooth muscle cells. *Atherosclerosis* **208**:56-61; 2010.
- [280] Cipollone, F.; Mezzetti, A.; Fazia, M. L.; Cuccurullo, C.; Iezzi, A.; Uchino, S.; Spigonardo, F.; Bucci, M.; Cuccurullo, F.; Prescott, S. M.; Stafforini, D. M. Association between 5-lipoxygenase expression and plaque instability in humans. *Arterioscler. Thromb. Vasc. Biol.* **25**:1665-1670; 2005.

Figure caption

Figure 1. Inflammation-dependent progression of the atherosclerotic process: putative differential involvement of the various LDL lipid oxidation products and of arachidonic acid derivatives.

Abbreviations used: oxPLs, oxidized phospholipids; lyso-PC, lysophosphatidylcholine; HNE, 4-hydroxy-2-nonenal.

I - ENDOTHELIAL CELL INJURY AND DYSFUNCTION

- adhesion and migration of circulating blood cells (monocytes, lymphocytes, platelets, ..)
- infiltration of plasma lipids into the arterial intima



II - ATHEROMA INITIATION

- monocyte and smooth muscle cell migration in the subintimal space
- fibrous cap formation
- cell differentiation and foam cell formation



III - ATHEROMA PROGRESSION

- monocyte and smooth muscle cell sustained cross-talk
- smooth muscle cell proliferation and extracellular matrix deposition



IV - ATHEROMA INSTABILITY AND RUPTURE

- apoptosis of vascular cells
- extracellular matrix degradation
- fibrous cap demolition

oxPLs
prostanoids
leukotrienes
lyso-PC
oxysterols
isoprostanes

oxysterols
HNE
leukotrienes
prostanoids
isoprostanes
oxPLs
lyso-PC

oxysterols
HNE
core-aldehydes
prostanoids
isoprostanes
leukotrienes

oxysterols
prostanoids
isoprostanes
leukotrienes
oxPLs
HNE